A LABORATORY GUIDE

FOR

GENERAL BOTANY

BY

C. STUART GAGER

DIRECTOR OF THE BROOKLYN BOTANIC GARDEN

THIRD EDITION

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PREFACE TO THIRD EDITION

The call for a Third Edition has afforded an opportunity of correcting a few minor errors in the Second Edition, and of making several additions and revisions. No new plant-types nor exercises have been added.

The author is particularly indebted to Professors L. R. Hesler and H. M. Jennison, of the University of Tennessee, for numerous constructive suggestions.

The fact that this LABORATORY GUIDE is entering a Third Edition would seem to indicate that many college teachers agree with the pedagogical principles briefly stated in the Preface to the first edition.

C. STUART GAGER.

BROOKLYN BOTANIC GARDEN,
January 20, 1926.

PREFACE TO SECOND EDITION

At the suggestion of teachers who have been using the first edition, laboratory directions are added for the study of three additional forms, viz.: *Exoascus deformans*, *Microsphæra Alni*, and *Ustilago Zeæ*. The author is indebted to Dr. E. W. Olive for the preparation of these directions, and for a careful re-reading of the entire text, resulting in the correction of various typographical errors and other inaccuracies.

The directions under the caption, VII, The Path of Water in the Plant (B–F, pp. 35–41), have been con-

The author is indebted to Dr. E. W. Olive for his careful reading of portions of the page proof.

C. STUART GAGER.

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CONTENTS

A LABORATORY GUIDE FOR GENERAL BOTANY

TO THE STUDENT

THE NATURE AND PURPOSE OF LABORATORY WORK

A. The Laboratory:

1. The word laboratory is derived from the Latin word *labor*, meaning *work*. A laboratory, therefore, is a workshop. The essential part of laboratory work, however, is not the manual but the intellectual. Handling specimens, manipulating apparatus, taking notes, and making drawings, all are essential, but are wholly secondary to thinking. *A laboratory exercise should be regarded always and primarily as a thought exercise.* Everything else that you do with a specimen should be secondary to thinking about it, and done only to aid thought.
2. The aim of laboratory work is to obtain facts at first hand. Reading books on plants is only studying about botany. To study botany one must have the actual plants before him. It was Louis Agassiz who said, "If you study nature in books when you go out of doors you cannot find her." The possession of this first-hand knowledge makes the reading of botanical books not only more easy, but vastly more interesting. You can take more away from the text because you bring more to it.

3. Another aim of laboratory work, not less important than the one just mentioned, is to acquire scientific habits of thought and work; to learn the method by which knowledge of the given science is acquired. The scientific method differs from the unscientific in laying emphasis upon the absolute necessity of an orderly procedure in thinking and doing, upon willingness to put aside prejudice and preconceived notions, upon scrupulous neatness, accuracy of thought and work, and careful attention to minute details. The scientific method is not peculiar to the natural sciences: it is just as essential in history or language-study as elsewhere, and the highest success in any intellectual pursuit is not possible if the requirements of the scientific method are disregarded.

B. Observation:

4. Observation is not merely looking at a thing. It means looking for a purpose. The mental attitude of the true observer is that of a questioner. The great Swiss botanist, de Candolle, said, **"The interrogation point is the key to all the sciences."** Observation, then, consists in asking as definite questions as possible about natural objects, and seeking their answer, not from the instructor nor the text-book, but from the object itself.

5. Remember that your specimen is the final authority in all matters of fact. Your first question should never be, "What *ought* I to see?" "How many parts *ought* the specimen to have?" but always, without exception, "What *do* I see?" "How many parts *does* the specimen have?" Possibly your specimen may be found to differ from that of your neighbor, or from the descriptions in the books.

If so, record that fact, and endeavor to ascertain whether your specimen is abnormal, or whether your observation of it is at fault in any way. *Always try to see all you can with the unaided eye before resorting to the aid of a hand lens or microscope.*

C. *Experimentation:*

6. In mere observation one takes conditions as he finds them; in experimentation, he determines, within limits, the conditions under which the observation is made. It is never possible to control, absolutely, all the conditions in any experiment, but this is partly compensated for by arranging side by side of the experiment proper, a check or *control.* In the experiment and control all conditions should be as nearly alike as possible save one. *The golden rule in experimenting is: vary only one condition at a time.* Then if the experiment and control give unlike results, we are justified in attributing the difference to the unlike factor.

7. Before beginning an experiment, the object, or aim, of the experiment must be clearly conceived and clearly stated. The necessary materials and apparatus should next be decided upon and procured. Then may follow the operation, that is, the arrangement of the materials and apparatus in a suitable way. This step is frequently referred to as "setting up" the experiment. The record of it should include an accurate statement of the conditions at the beginning of the experiment, together with drawings of the apparatus and material as the experiment is set up.

Next follows the observation, which *must always be made and recorded at the time and place of the experiment.* It should include suitable drawings.

Finally, there may be stated the inference, that is, the conclusion or conclusions which are thought to be justified by the facts observed.

The record of an experiment, then, should follow the outline given below:

1. Object.
2. Materials and apparatus (with drawings).
3. Operation.
4. Observation (with drawings).
5. Inference.
6. Remarks.

D. The Note-book:

8. The Note-book serves two purposes: First, the making of it gives you opportunity to acquire facility in describing what you observe. This is not an easy accomplishment, but a very essential one. "*The greatest thing a human being ever does in this world,*" said John Ruskin, "*is to see something, and tell what he saw in a plain way.*"

9. Secondly, the note-book serves as an index, to the instructor, of what you have done and how well you have done it. In addition to these two purposes, the note-book will be a permanent record for your own future use. It should contain a complete record of all you observe, and the inferences you make from these observations. It should include written descriptions and drawings. In both the latter the aim should be accuracy, neatness, completeness, conciseness. Above all things, it should be a record of your own observation, not of your neighbor's. If, as may happen on rare occasions, it becomes necessary to use your neighbor's notes, always state the fact clearly and frankly in your own book.

10. In writing your notes, the aim should be to give such a clear account of what you have seen and done that anyone else who knew nothing of the subject could profit by reading them. In other words, *aim to make your notes usable in the future.* Your text-book may be regarded, in one sense, as the author's laboratory note-book. Seek to make your laboratory note-book an accurate and readable illustrated text on the ground covered by your course.

E. Laboratory Drawings:

11. Drawing is one of the greatest aids to observation. This is its main purpose in the laboratory. It is often said that "persons who cannot draw cannot see." This is probably an extreme statement, but it is undoubtedly true that one who can make an accurate drawing of a thing has observed it more accurately than one who cannot.
12. Laboratory drawings should aim to represent the thing only as it is, not as it may impress one at first sight. They differ in this respect from the work of the artist. For example, to show the exact number and location of the veins of a leaf would ruin the artist's picture; but without those details the laboratory drawing would be of little value.
13. As directed in the GUIDE, make as thorough an observation of the object as possible before you begin to draw; then make the drawing.
14. Unless otherwise directed, make outline drawings, shading only where absolutely necessary. In general, *every line in your drawing should represent some fact of structure in the specimen.*

15. Be sure to make the drawing large enough so that all details may be included without crowding or confusion.
16. First sketch in the outline lightly with a 5H drawing pencil. In finishing a 2H pencil may sometimes be desirable.
17. All drawings should be on unruled sheets, and only on one side of the sheet. They should be labeled and numbered consecutively throughout the course by writing under each the abbreviation *Fig.*, followed by the proper numerals, and then by the legend or label, stating what the object is, and what view of it is shown, as for example, "Cross-section, end view." Each drawing should have all of its essential parts labeled by extending straight horizontal dotted lines from the various parts (using a ruler), and writing the name of the part at the end of the line.
18. The arrangement of the drawings on the page should receive careful attention, so as to make as attractive and well balanced a page as possible. *Crowding should be avoided*, and on any one page should be included only those drawings that represent parts of the same plant, or pertain to the same subject.
19. The various pages of drawings should be numbered and labeled near the top of the page at the middle thus; *Plate I*. Throughout your written notes, when describing a structure or apparatus represented by a drawing, refer to the drawing by its proper number and the number of the Plate (*e.g.*, *Plate IV*, *Fig.* 5).
20. At the completion of the course, arrange a "*Table of Contents*," listing the main topics, as indicated

in the LABORATORY OUTLINE, in the order in which they occur in the note-book, with the page number near the right-hand edge, and a neat dotted line extending from the subject to the page number.

F. *The Microscope:*

21. Full directions for the use and care of the compound microscope will be given by the instructor. *The student should clearly realize from the first that the science does not reside in the instrument.* The latter is merely an aid to the eyes, but not to the mind, and is made necessary by the limited range of our unaided vision. It should be used only after one has seen all that he possibly can with the unaided eye.

22. The following points should be constantly borne in mind:

(*a*) Keep all parts of the instrument, especially the lenses, scrupulously clean.

(*b*) Never attempt to take the instrument apart.

(*c*) Never remove lenses from the stand. If it is ever absolutely necessary to do so, then

(*d*) Never lay a lens down on the table.

(*e*) Never touch the lens with the fingers or eyelids.

(*f*) *Never try to clean the lens with the handkerchief or anything except lens paper.*

(*g*) Never examine any object without covering it with a cover-glass.

(*h*) Never allow the object to touch the cover-glass.

(*i*) *Never focus down while looking through the microscope.*

(*k*) Be sure that the slides and covers are absolutely clean. Dirt will be magnified as well as the object you are studying.

(*l*) Handle all slides and cover-glasses by the edge, never touching their surface with the fingers.

(*m*) Don't shut one eye when looking through the instrument. Ability to work with both eyes open is easily acquired, is much less tiring, and is an advantage in many ways.

(*n*) *Never use high powers when low powers will serve.*

(*o*) Examine all objects with the low power first, then with the high power, if necessary.

(*p*) Never set the instrument away with a microscopic slide under the objective, nor with the high-power objective over the aperture.

(*q*) When the laboratory period is over, remove the preparation you have been studying, and leave the microscope either with the low-power objective over the aperture, or, if a dust-proof nose-piece is used, then with the objectives sidewise.

PART I

ANATOMY AND PHYSIOLOGY

I. Meaning of the Terms

A. **Plant physiology is that branch of botany which deals with the vital activities of plants.** But physiological processes or functions are carried on by various parts of the plant, and these parts all have their own characteristic structure. In order to understand the processes we must know the internal as well as the external structure of the parts concerned. This knowledge requires dissection, and this phase of the science is, therefore, called **anatomy.** Microscopic anatomy is called **histology.** Just as the processes cannot be intelligently considered apart from the structures involved, so, also, the study of anatomy apart from physiology is meaningless.

B. In the lowest (*i.e.*, most simply organized) plants all functions, both nutritive and reproductive, are performed by every structural unit or cell; but in more highly organized plants there are special parts or organs for the performance of each function; for example, roots to take in moisture, flowers to form seed. In other words, in the higher plants there is a **division of physiological labor,** or, as it is sometimes called, a physiological division of labor. While not entirely wanting, the division of physiological labor is less marked in the lowest plants.

Because they are composed of organs, plants and animals are termed **organisms.**

C. Thus we see that some plants have a generalized plant-body, others a more highly specialized one. A generalized plant will be studied first, then the structure of a higher (*i.e.*, more highly specialized) plant. This will be followed by an elementary study of the fundamental life-processes involved in the nutrition and growth of the individual. The second part of the course will be devoted to studying the various kinds of plants, and the numerous ways in which different kinds of plants solve these same life-problems of nutrition and reproduction.

II. A Generalized Plant (*Spirogyra*)

A. Naked-eye Characters:

1. Carefully take a small bit of this plant between the thumb and fingers and note its "feel." Suggest why it is sometimes referred to as "green silk."
2. Carefully lift up some of the material with a needle, and describe the form of the plant. How many centimeters long are the longest filaments you can observe?
3. Can you detect any evidences of a **differentiation** of the plant into **shoot** (*i.e.*, stem and leaves) and **root?**

B. Microscopic Characters:

1. *The plant as a whole.*
   (*a*) Mount two or three filaments in water.
   (*b*) Note the filament is composed of separate structural units, placed end to end. These units are **cells.**
   (*c*) Are the filaments more than one cell thick? Do they branch? Are they of uniform diameter? Compare the length of the various cells with each other. Compare the shape of the end cell with that of the others. What is the shape of the filament as seen in imaginary cross-section? Very careful focussing is necessary in order to answer this question correctly.
   (*d*) Accurately measure the length (in millimeters) of a piece of filament lying straight under the cover-glass, then count the number of cells in

this piece. Calculate the average length of the cells, and the number of cells in the longest filament observed. Estimate the length of an individual cell in terms of its diameter, and from this calculate the diameter of the filament.

(*e*) Using the low power and removing the cover-glass, carefully cut a filament apart with the scalpel, causing a little injury as possible. As you do this observe the exposed end-walls of the uninjured cells that now terminate the filament where it was broken apart. Describe and try to account for what you see. Is there any evidence of the existence of a force within the cell? If so, in what direction does it act? Make two outline drawings, showing the conditions before and after cutting.

(*f*) Make a diagram about 75 mm. long, illustrating the outline of the three terminal cells of a filament, as seen in optical section. Omit all details of cell-structure.

2. *The individual cell.*

(*a*) Center your attention on any one of these cells, and identify the following organs of the cell:

(1) A **cell-wall,** enclosing all other parts of the cell. Is it transparent or not? Give a reason for your answer. Note its relative thickness. The wall is composed of **cellulose.** Has each cell its own end-wall, or is there a common end-wall for two adjacent cells?

(2) The substance enclosed by the cell-wall is largely living matter, or matter in the living state. It is called **protoplasm.** The unit of protoplasm of each individual cell is

called a **protoplast.** Distinguish the following parts of a protoplast:

(3) The prominent green chlorophyll-band, or **chromatophore.** Describe its form, the number of turns it makes in the cell, and the outline of its **margin.** Infer its shape in cross-section. How many bands in each cell? If more than one, do they coil in the same direction? Can you detect free ends of the chromatophore? Are they continuous from cell to cell? The color of the chlorophyll-band is due to the presence of a green pigment, **chlorophyll.**

(4) The denser areas within the chromatophore are regions of starch-formation. In the center of this area is the starch-forming body, or **pyrenoid.** Surrounding the pyrenoid are starch grains.

(5) Make a detailed drawing, 10 mm. wide and 15 mm. long, showing the details of structure of a portion of the chlorophyll-band, as seen under high power. Indicate on the drawing the names of all parts shown.

(6) At or near the center of the cell find a dense, colorless body, the **nucleus,** surrounded by a less dense layer of colorless **cytoplasm.** Describe the shape of the nucleus. From the layer of cytoplasm trace

(7) Delicate **cytoplasmic strands,** extending to the pyrenoids, and to

(8) The **lining layer of cytoplasm.** This layer (sometimes called "primordial utricle") is in intimate contact with the entire inner surface of the cell-wall, and is difficult to

III. A Specialized Plant (*e.g., The Bean Seedling*)[1]

A. The plant as a whole:

1. Examine the seedling given you and note that it is composed of an **axis** with appendages; the axis, of **root** and **shoot;** the root, of a **primary root** with branches (**secondary roots**); and the shoot, of a main **stem,** bearing **leaves.** Has the main stem **branches?** Is this true of all plants? What is the difference between a stem and a branch?
2. Describe fully the location of the leaves and their attitude on the stem. Do they occur on both the main stem and its branches? The places on the stem where leaves grow are **nodes.** The spaces between the nodes are called **internodes.**
3. Compare the size of the upper with that of the lower angle made by the leaves with the stem. This upper angle is called the **leaf-axil** (Latin *axilla,* armpit).
4. Do you find any structures in the leaf-axils? If so, describe them. What are they?
5. With what do the tips of the main stem and branches terminate?
6. Describe any other outgrowths of stem or branches.
7. Make a drawing of the plant as large as your drawing paper will permit, showing all parts referred to above.

[1] This study may or may not be omitted, depending upon the previous preparation of the students, and the time available.

IV. Structure of the Foliage-leaf (*e.g., Lilac leaf*)

A. External Characters:

1. Make a drawing, natural size, showing all the parts of the foliage-leaf given you, as seen from the under side.
2. Identify the following parts, and label them suitably on your drawing:
   (*a*) The flat, expanded **blade.** Describe its **coloration** (*i.e.*, the kind and distribution of color). Is the blade **simple** (*i.e.*, not divided into leaflets), or **compound** (*i.e.*, branched, divided into leaflets)? The surface that lies uppermost, as the leaf bends back from its position in the bud, is the **ventral** surface; the under surface is the **dorsal** one. These terms are applied with reference to the position of the leaf in the bud.
   (*b*) The **leaf-apex,** which is also the apex of the blade.
   (*c*) The **margin** of the blade.
   (*d*) The **base of the blade.**
   (*e*) The **venation**(distribution of veins in the blade). Describe it as parallel-veined, pinnately netted-veined (with a **midrib**), or palmately netted-veined. Describe the difference between the three types of venation. Is there a **marginal vein?** If so, suggest what advantage it may be to the leaf.
   (*f*) The **petiole** (stem of the leaf). Leaves having a petiole are **petiolate,** otherwise **sessile.**

(*g*) The **leaf-base,** the portion by which the leaf is attached to the branch.
(*h*) If present, the **stipules,** outgrowths of the leaf-base. Leaves without stipules are **exstipulate.**
(*i*) Before the next class exercise compare with the leaf studied, as directed above, various other types of leaves collected by yourself, making full drawings and notes.

B. Anatomy of the Leaf:

THE LOWER EPIDERMIS[1]

1. As directed by the instructor, remove a strip of the lower epidermis of a foliage-leaf, and mount it in water or clearing fluid, being sure to have the outer surface uppermost. Record the name of the species.
2. Note the cellular structure of the epidermis. A group of cells, similar in structure and function, is called a **tissue.** The leaf-epidermis is epidermal tissue. The cell-wall forms a box, having depth as well as length and breadth. Note that you see only the edges of the vertical walls. How many walls altogether, bounding each cell? Are they visible? Explain. Are the cell-walls transparent or opaque? Give a reason for your answer. Suggest the advantage of this feature to the plant. Make a diagram of an epidermal cell as seen in perspective.
3. Observe the somewhat lenticular openings, or pores, each surrounded by crescent-shaped cells.

[1] MISS ECKERSON (*Bot. Gaz.*, 46: 221–224. S1908) has recommended the leaves of the following plants as specially satisfactory for the study of the epidermis and stomata: Sunflower, *Fuchsia speciosa*, zonal Geranium, and *Tradescantia zebrina*

The openings are **stomata** (Latin singular, **stoma,** *a mouth*). The crescent-shaped cells are **guard-cells.** How many has each stoma?

4. Do the guard-cells and other epidermal cells contain chlorophyll-bodies (**chloroplasts**)? Describe their shape. They are not considered identical with the chlorophyll-band of *Spirogyra*, hence the different name.
5. Note the shape and arrangement of the other epidermal cells. Are they in the same plane as the guard-cells? Describe, giving reasons for your answer.
6. State the number of stomata visible in the entire field (high power). Record three counts, each of a different area, and the average. Why is this desirable? After ascertaining the area of the *lens* of the objective of your microscope, calculate, from several counts, the average number of stomata per square centimeter.
7. Make a drawing showing at least three stomata with their guard-cells and adjacent epidermal cells. The guard-cells should be at least 15 mm. long.

THE UPPER EPIDERMIS

8. As directed in *B*, 1–6 above, study the structure of the upper epidermis of the same leaf. Draw.
9. Compare the structure of the upper with that of the lower epidermis, noting, among other features, the relative number of stomata in each.
10. In the light of the experiments on transpiration,[1] what do you think is one function of these stomata? Of the guard-cells?

[1] This takes for granted that class demonstrations of transpiration have been given.

11. Give one explanation of the difference in the rate of transpiration from the two surfaces of the leaf. Is this the only explanation?

CROSS-SECTIONAL VIEW

12. Mount thin free-hand sections of a fresh leaf showing the internal anatomy as seen in cross-section.
13. Identify in your section the two epidermal layers. How many cells thick are they? Do you find any chloroplasts in these layers?
14. Are all the epidermal cell-walls of the same thickness? Describe any variations observed.
15. Is there a thick, continuous pellicle over the surface of the leaf? Is it composed of cells? Such a pellicle, when it occurs, is called **cuticle,** and is composed of a waxy material, **cutin.**
16. Compare the thickness of the cell-walls in the upper and the lower epidermis.
17. Note the stomata and guard-cells, and their relation to the other epidermal cells.
18. The tissue between the two epidermal layers is composed chiefly of leaf-parenchyma, or **mesophyll,** in which are imbedded the veins. Mesophyll, and all other tissue containing chlorophyll, whether found in leaves or in other organs, is also called **chlorenchyma.** Note that the mesophyll is composed of two distinct groups of cells, as follows:
19. The more compactly lying cells beneath the upper epidermis compose the palisade layer, or **palisade parenchyma.** Describe their shape, contents, relative size, and relation to each other and to the epidermis.
20. Between this layer and the lower epidermis lies the **spongy parenchyma.** Describe its appearance, and

the cells that compose it. Compare it with the palisade layer.

21. What fills the space between the mesophyll-cells? Do these spaces connect with the outside air? If so, how?

THE VEINS

22. At certain regions the section passes through veins, presenting either cross, longitudinal, or other sections of them. Note the greater differentiation of the cells in the veins. This differentiation marks the distinction between fundamental tissue or **parenchyma,** and transformed, elongated tissue, **prosenchyma.** There are several different kinds of prosenchyma.

23. Using prepared slides, supplied by the instructor, make a drawing of the cross-section of a leaf, showing all features noted above. Make the drawing at least 75 mm. long, and be careful to preserve the natural proportions.

V. TRANSPIRATION[1]

A. Loss of Weight of a Growing Plant:

Experiment 1.—Object:[2] To show that a living plant is constantly losing weight.

1. Choose a well-watered, vigorous, potted plant. Wrap the pot in sheet-rubber or oilcloth (or paraffined paper), and tie the wrapping about the stem tightly, but not tightly enough to cause injury. Place the plant thus prepared on a pair of balances in a well-lighted window, and record its exact weight in grams in the following table, which should

Time		Weight in grams
Day	Hour	

be copied into your note-book. After the weighing, the window should be opened (if the weather is not too cold); direct sunlight is also desirable. Record

[1] NOTE.—Where the class is large, or the laboratory equipment limited, and especially when the course extends over only one semester, it is recommended that most, if not all, of the physiological experiments outlined in the remainder of Part I be performed by the instructor as demonstrations in the presence of class.

If desirable, in order to secure better light conditions, the subject of Nutrition (pp. 45–55) may be taken up next, returning then to transpiration, etc. (pp. 22–44).

[2] For directions for recording an experiment see p. 4 of this GUIDE. In each experiment this outline is to be filled out entire, without further directions.

the weight at five or six successive periods, and then, as directed by the instructor, plot on section-paper a curve of your readings. Lay off the observed weights as ordinates, the time-intervals as abscissæ. *Be sure that in this and all subsequent experiments your inferences are only those warranted by your observations.*

Experiment 2.—To ascertain one cause of loss of weight of plants.

1. Take four clean, dry, glass beakers or tumblers, two pieces of cardboard large enough amply to cover the opening of the beaker, and a vigorous green leaf *having a leaf-stalk and a perfectly dry surface.*
2. Fill two of the glass beakers or tumblers three-fourths full of water, insert the leaf-stalk through a small hole in the center of one piece of cardboard, make the opening as tight as possible about the leaf-stem, using cotton if necessary, and place the cardboard over one of the water-containing beakers, so that the leaf-stalk extends down into the water. Invert one of the dry beakers over the leaf. Arrange the other two beakers and cardboard in the same way, only omitting the leaf and the hole through the cardboard. This second set of beakers is the **control** (*cf*. p. 3, ¶6).
3. Place both sets of beakers in a well-lighted window, preferably in direct sunlight, and from time to time observe and compare the appearance of the inner surfaces of the inverted beakers.
4. Do you notice any difference in the result on opposite sides of the leaf? If so, describe.
5. Can you see any water passing from the leaves? In what state, therefore, does it pass off? From what part of the leaf does it come? Why do you

think so? What change does it undergo in order to become visible on the surface of the beaker? In what state does it probably exist in the leaf? State one reason why the plant lost weight in *Experiment* 1. Was this the only cause of its loss of weight?

6. The above experiments demonstrate the fact of **transpiration.** Give a definition of transpiration.

B. *The Control of Transpiration:*

Experiment 3.—To see if the epidermis affects the rate of transpiration.

1. Take two sound apples. Remove the skin (epidermis) from one of them, then ascertain accurately and record the weight of each, in tabular form, as follows:

Time		Weight in grams	
Day	Hour	Unpared	Pared

2. Place the specimens in a convenient place, with free access of air, and out of reach of mice.
3. Record, in a table like the above, three (or more) subsequent observations of weight at successive class periods.
4. Plot two curves showing the rate of loss of weight. Include these curves and their interpretation as part of your record of the experiment.

Experiment 4.—To demonstrate the effect of the "skin" of a potato-tuber on transpiration.

1. Proceed as directed for *Experiment* 3, using two sound potatoes instead of apples.
2. The "skin" of an apple is a true **epidermis,** having an outer layer of **cuticle,** which is not readily permeable by water. The "skin" of a potato-tuber consists of several layers of cork cells. It is this corky tissue which chiefly retards the loss or water from the tubers.

Experiment 5.—After noting the color change caused by wetting dry cobalt paper (prepared by dipping filter paper into a solution of cobalt chloride and thoroughly drying it), make the following experiment: Place discs of the cobalt paper (*e.g.*, as large as a five cent piece) on opposite sides of a lilac leaf having *dry surfaces*, and hold all in place between two microscopic slides (or larger pieces of glass), fastened with rubber bands around each end. Compare the rate of color change of the two opposite discs, and infer the cause.

Other leaves, having structural peculiarities similar to those of the lilac, may be used; *e.g.*, hibiscus, osage orange, oleander, lizard's tail (*Saururus*).

Experiment 6.—Examine, with the microscope, strips of both upper and lower epidermis of the leaf used in *Experiment* 5, and infer the probable cause of the differential color-change observed.

Experiment 7.—Place any suitable, well-watered potted plant on postal scales, "household" scales, or other convenient weighing device, after first carefully wrapping the pot in sheet-rubber, or sheet-oilcloth, as in *Experiment* 1. Record the loss of weight at fifteen-minute (or other suitable) intervals while

the experiment is standing for an hour, each, in (*a*) direct sunlight and breeze; (*b*) diffuse sunlight, and the comparatively still air of a room; (*c*) under a glass bell-jar, or large box. The breeze may be secured by placing the experiment in or near an open window or other draught, or by means of an electric fan.

On the basis of your observations in *Experiment* 7, discuss the control of transpiration by external conditions, and suggest differences in the condition of the plant caused by its exposure in the various situations suggested above, and the effect this would have on the rate of transpiration. Were the results observed in the three situations strictly comparable? Why?

C. *One Effect of Transpiration:*

Experiment 8.—To show the so-called "lifting power" of transpiration.

1. Insert a leafy stem of a living plant (a branch of any evergreen is excellent to use) into one end of a piece of glass tubing about 3 ft. long, of small bore, and full of tap-water, taking special care to have the joint between the stem and the glass air-tight, using rubber tubing for this purpose if necessary. The experiment will be more satisfactory if the stem is cut off under water, and the cut end kept from contact with air, throughout the experiment.
2. After being sure that the glass tube is full of water, place it upright in a dish of mercury, having care not to allow any of the water to run out in so doing.
3. Place the experiment in sunlight, if possible, but do not leave it in direct sunlight for more than one-half to three-quarters of an hour.

4. At the beginning of the experiment, and at suitable intervals thereafter, as directed by the instructor, measure and record the height of the mercury in the glass tube.
5. Make two drawings of this apparatus in longitudinal section: (*a*) as soon as the experiment is set up; (*b*) at the close of your final observation. Label all essential parts.
6. You have made this last experiment with a living plant. The question now naturally arises: Is the result observed due to the life-factor involved, or is it merely the result of some physical condition, as, *e.g.*, the evaporation? The question may be easily answered by setting up an experiment similar to the preceding, but using non-living material, as follows:

Experiment 9:—To see if evaporation exerts a "lifting power."

1. Tie a piece of porous animal membrane (*e.g.*, bladder) over a thistle-tube, being sure that there is no chance for a leak between the glass and the membrane.
2. Fill the thistle-tube with water.
3. Prepare a dish of mercury and also a clamp to hold the tube in place.
4. Invert the thistle-tube and place the lower end in the mercury, being sure that no air enters the tube. By this arrangement all factors of *Experiment* 8 have been eliminated except evaporation, and the evaporation takes place through only one membrane, and that a non-living one. In other words, we have *Experiment* 8 reduced to its lowest terms.
5. Observe and record the heights of the mercury in the tube as in *Experiment* 8.

6. Make a drawing of this apparatus in longitudinal section at the beginning and at the close of the experiment, labeling all essential parts.
7. The conclusions drawn from this experiment should cover an explanation of the bearing of these results on *Experiment* 8.
8. Does evaporation (or transpiration) lift the water? If not, what forces the mercury to rise in the stem of the thistle-tube? Can a similar force operate to cause sap to rise in trees even to a height of 100 feet or more? Do *Experiments* 8 and 9 afford an adequate explanation of the ascent of sap? Briefly discuss this point.
9. The experiments on transpiration have shown that living plants are constantly losing water. What would be the result if no more were supplied? What problem of plant life, therefore, naturally arises?

VI. Absorption of Water by Plants

A. External Anatomy of the Root:

1. Examine roots of seedlings (mustard, flax, oats, etc.), grown in a moist chamber (*e.g.*, flower-pot, or saucer of same) in the dark, and kept covered with a glass plate so as to expose them to the air as little as possible. Note the delicate white hairs on them. Describe their distribution and relative size. These hairs are **root-hairs.**
2. Hold the root up to the light and note the more transparent tissue on the end (**root-cap**), covering the **root-tip** proper. How is the latter distinguished? Is "root-tip" synonymous with "end of the root?" Explain.
3. Make a drawing of the seedling, at least twice natural size, showing these features. (The labeling of the root-cap and root-tip may be deferred until observation *B*, 3, below, has been made.)

B. Microscopic Characters of the Root:

1. With the scalpel carefully remove the terminal 5 to 6 mm. of a root, with the root-hairs, and mount it in water. Locate the oldest and youngest root-hairs. How are they distinguished? Do they branch? What relation do the hairs bear to the epidermis? Are they divided by cross-walls? Do they contain nuclei? What is a root-hair, structurally?
2. Make a drawing (high power) of three or four hairs, showing their structure and relation to the epidermis. The hairs should be drawn at least 50 to 75 mm. long.

3. Distinguish the root-tip from the root-cap. Of what is the latter composed? Describe it. Draw.

C. *The Function of Root-hairs:*

1. Carefully pull up a mustard seedling growing in sand and having several leaves. Without injuring the plant, carefully and very gently shake off all sand that readily falls away. Does the sand adhere with equal firmness to all portions of the root? Describe in detail, explain, and illustrate by a drawing, x2.
2. Pull up another seedling of the same age, and remove all or most of the adhering sand. Replant both seedlings in sand, water them, and set them aside until the next period. *In order to eliminate individual differences it is necessary to treat several seedlings in each of the ways above indicated.*
3. At the next meeting of the class observe and compare the appearance of the seedlings. In thoroughly removing the sand from the seedlings, how were the root-hairs affected?
4. By means of what organs does a land plant obtain most of its water? State, in a paragraph, the reasons for your answer.
5. We have ascertained the organs whose function it is to take in water from the soil. It is now important to inquire by what process the soil-water passes into the plant through the organs of absorption.

D. *How the Root-hairs Take in Water; Osmosis:*

1. The preceding studies of the plant cell lead us to recognize the fact that the root-hair is a branch of the epidermal cell. Within is the cell-sap, a weak solution of various salts, sugars, and other substances; without, as the plant grows in the soil, is

the soil-water, also containing numerous substances in solution. The cell-sap of the root-hairs and the soil-water are solutions of different densities, and separated by layers of porous (semipermeable) plant substance. Name these layers.

Experiment 10.—To see what results when two liquids of unequal density are separated by a porous membrane:

2. With a pen knife or a pair of scissors, remove a portion of the shell from the large end of a hen's egg, taking great care not to puncture the membrane that separates the white of the egg from the shell.
3. Carefully place the egg thus prepared upright in a glass tumbler, or beaker, and pour in tap-water until the water surface is about 1 in. above the egg.
4. By the above arrangement the solution of various salts intermingled with the substance of the egg serves as the most dense liquid, the water outside as the less dense, while the membrane in the egg acts as the porous membrane, separating the two liquids. In other words, we have roughly imitated the plant cell, though there is nothing in the cell that corresponds to the shell of the egg.
5. Make a careful drawing, showing the experiment in longitudinal section, and about one-half natural size. Label all parts.
6. Make an observation at the end of an hour; of two hours. Describe what results, and illustrate the final result by another sectional drawing opposite the first one.
7. State as clearly as you can what has taken place in order to produce the result observed. The process is termed **osmosis** (Greek, *osmos*, pushing).

8. In this experiment, what part of the root-hair does the egg membrane represent? the solution of salts in the egg? the water in the beaker?
9. From the above study explain what takes place when a root-hair is in moist soil. What is thus accomplished for the plant?
10. Define osmosis.

Experiment 11.—To demonstrate osmosis in a plant cell:

11. Mount in water several uninjured root-hairs. Again identify (high power) the lining layer of cytoplasm. Make a drawing, about 50 mm. long, of one of the root-hairs. Leave room for two other drawings by the side of the first one. Run a drop of a 5 per cent. salt solution under the cover-glass. This solution is more dense than the cell-sap.
12. Describe the effect of the salt solution on the protoplast.
13. Make a drawing by the side of the first one, showing what you observe. What is the process called?
14. Now thoroughly irrigate the cells with fresh water, and observe and describe the results. Explain as fully as you can.
15. By the side of your second drawing make a third, showing the cell as it appears after irrigation with fresh water.
16. In a sentence, name, in order, two processes that take place, (*a*) when a living plant cell is immersed in a solution more dense than the cell-sap; (*b*) when a plasmolyzed cell is irrigated with tap-water.
17. What is one function of the substances dissolved in the cell-sap? What is one function of the plasma membrane?

Experiment 12.—Demonstration of the **Osmoscope** (by the instructor).*

18. Make a drawing showing clearly all essential parts as seen in longitudinal section, and describe the apparatus as set up and explained by the instructor.
19. Record observations on the height of the column of water in the tube of the osmoscope:
    (*a*) At the beginning of the experiment.
    (*b*) On successive half-hours.
    (*c*) On successive days.
20. Explain the results observed.

Experiment 13.—Demonstration of "exudation-pressure" (by the instructor).

21. Describe and make a drawing of the experiment at its beginning, as set up by the instructor.
22. Complete your observations and record as directed under *Experiment* 12, naming the species of plant used.
23. Compare the conditions and results in this experiment with those in *Experiment* 12.

* It is here taken for granted that the instructor will be able to make this demonstration (as well as that under *Experiment* 13) without further suggestions, using any one of the various types of osmoscope commonly found in botanical laboratories.

VII. The Path of Water in the Plant: Structure of Stems*

A. The Function of the Stem:

Experiment 14.—To see if there are definite channels for the passage of liquids through a stem.

1. Place the cut ends of various living, leafy shoots (*e.g.*, corn, plantain, lily leaves, parsnip, or seedlings of castor-oil plants), into an aqueous solution of eosin, and, after they have stood for a suitable time, as determined by the instructor, observe *freshly exposed* end-surfaces, and note the regions where the colored solution appears. Does it pass up through the whole mass of tissue, or are there definite channels through which it rises? Cut sections of the stems at various heights, and observe and describe the distribution of the colored areas.
2. Compare the distribution of the colored areas in a parsnip (or seedling of a castor oil plant) and a stalk of corn (or petiole of some lily leaf). Make a diagram to illustrate this.
3. Examine the end of a dry corn stalk, and note the projecting strands. What relation do they bear to the paths of the eosin? They are composed of fibers and vessels united, and are therefore called **fibro-vascular bundles.** To what class of tissue do they belong?
4. Carefully cut *the epidermis* in a ring around the petiole of a leaf of plantain, being specially careful not to cut clear through the petiole.

* The outline for §VII, B–F (pp. 35–41) was prepared by Prof. Ernest Shaw Reynolds, Agricultural College, N. D.

5. Taking the end of the petiole in one hand and the leaf-blade in the other, gently pull the two portions of the petiole a short distance apart. Describe and illustrate by a drawing what you observe. What structures are thus disclosed?
6. What relation do the fibro-vascular bundles bear to the veins of the leaf? To the root-hairs?
7. Write a clear statement of how the water passes from the soil into the roots of a plant, and into and through the leaves and out into the air, mentioning, in order, all parts and processes studied.

B. Internal Structure of a Monocotyledonous (Endogenous) Stem:

1. Examine with your naked eye a thin cross-section of a corn stem. What general features are to be observed? What relation do the dots, seen in this cross-section, bear to the fibro-vascular bundles? What then are these dots? Now study the section under the hand lens and compare the number of bundles near the center and near the periphery. Where are they most numerous? What mechanical advantage do you see in the observed arrangement of the bundles? The cells intermixed with the bundles are *pith* cells. What kind of tissue? How far from the center do they extend toward the periphery?
2. Make a drawing of the cross-section, as seen under the hand lens, about 10 cm. in diameter, then examine the section under the microscope. Illustrate with a drawing at least 12 cm. wide,the cross-section of a vascular bundle located near the center of the stem. The yellowish ring of cells around the outside of the bundle is the *bundle sheath*. What features of the sheath make the color so noticeable?

The bundle sheath surrounds tissues in which there are three or four large open spaces: (1) The somewhat irregular space at one end is an *intercellular air passage;* (2) the two on either side of the middle are **tracheæ.** A ring from an *annular* **tracheid** may often be seen projecting into the inner end of the air passage; and one or more *spiral tracheids* may lie between the two large tracheæ. This group of tracheæ and tracheids constitutes the *xylem,* or water-conducting tissue. It is woody in consistency. The thin-walled, closely packed tissue, at the opposite side of the bundles from the air passage, is the **phloem** tissue, which under high power can be seen to be composed of two kinds of elements: (1) The larger cell-like spaces which are cross-sections of the **sieve-tubes;** and (2) the very small, nearly square cells, at the corners between the sieve-tubes, which are the **companion cells.** The phloem is concerned in the conducting of elaborated food, perhaps chiefly the nitrogenous foods. Compare the vascular bundles near the periphery with those at the center of the stem. What differences are there in structure, and in the relation of one bundle to another? How are the xylem and phloem placed in the bundle in relation to the periphery of the section?

3. Examine a longitudinal section of a corn stem of considerable length and make a diagram illustrating the course of the vascular bundles, especially noticing how and where the bundles (veins) from the leaf enter the stem and the course they take. What finally becomes of the leaf-bundles?

C. *Internal Structure of Herbaceous Dicotyledonous (Exogenous) Stems:*

1. Cut some very thin slices of any kind of wood and mount one piece in anilin sulphate and one, after it has been moistened with hydrochloric acid, in a solution of phloroglucin (warming the slide, if necessary, to develop the color). What colors are developed in the wood in each of the solutions? (Compare with one in water.) These color reactions are very characteristic of **lignified** (woody) cell-walls, although anilin sulphate also stains some fibers in other than wood regions.
2. Now cut thin cross-sections of some herbaceous dicotyledonous (exogenous) stem (*e.g.*, garden geranium). Mount these sections, some in anilin sulphate, some in phloroglucin after HCl, and some in water, for microscopic examination. These should show which parts of the herbaceous stem are lignified. Where are these lignified (xylem) portions, and how many are there in the stem? Was it easier to cut these or the wood sections? Why? The mass of tissue directly outside of each wood (xylem) region is the phloem tissue of the vascular bundle. Between the xylem and the phloem in each bundle observe a layer of one or two cells of **cambium,** an actively dividing tissue that produces more xylem and phloem. How are the bundles arranged in the stem? The tissue between the bundles and the periphery of the stem is composed of **cortex,** covered on the very outside with the **epidermis.**
3. Study these sections under the microscope and compare them with the corn stem. Do you find the same tissues in both cases, or with what exceptions?

How do they differ in arrangement? What would you consider the chief structural distinctions between the corn stem and this herbaceous stem?

4. Make a diagram of the cross-section as seen under the hand lens, labeling the tissues as thus seen. It is to be noted that monocotyledonous (endogenous) herbaceous stems are essentially like the corn stem in cross-section.

D. Primary Growth in a Woody Dicotyledonous (Exogenous) Stem:

1. Examine with a hand lens a cross-section of a young *Aristolochia*, or other woody stem, which has not yet developed tissue from the cambium layer. The arches of tissue near the periphery (in *Aristolochia*), as seen under the hand lens, form the outer boundary of the vascular bundles. How and where are the latter arranged? How many are there in the whole cross-section? In what general respects does this stem differ from that of the corn? How is it like the herbaceous stem? Make a diagram of the section.
2. Examine the section under the microscope. The bundles are seen to be somewhat wedge shaped. The inner part is the xylem, and the outer (toward the periphery) the phloem. The xylem is recognized by the large tracheæ. The cambium is also present. Where would you look for it? Make a careful drawing of a portion of the cross-section, including one complete bundle and at least a quarter of each bundle on each side; this will be a V-shaped sector with the point at the center of the stem-section and the arc at the periphery. Radiating from the center and between each pair of bundles are the **medullary rays.** How are these related to

the pith at the center? Into what tissue do they merge toward the periphery? The xylem is composed of the tracheæ, tracheids, and wood parenchyma. The phloem is similar to that of the corn stem. Outside of the ring of vascular bundles is the **pericycle,** and outside of that the **cortex,** both of the latter often becoming changed into dead bark eventually. On the very outside is the epidermis. Under the high power of the microscope determine how the epidermis is protected against excessive evaporation. About how many rows of cells are there in each of the tissues: epidermis, cortex, and pericycle?

3. Is there any fundamental difference between the young woody stem and an herbaceous stem? The arrangement and structure of the bundles in the corn stem are characteristic of the monocotyledonous (endogenous) type of stem, and the arrangement and structure of the bundles in the herbaceous and woody stems are characteristic of the dicotyledonous (exogenous) type of stem.

E. Secondary Growth of a Dicotyledonous (Exogenous) Stem:

1. Examine a thin cross-section of an *Aristolochia*, or other dicotyledonous stem, which is 3 or 4 years old. A hand lens view of this is to be drawn, indicating clearly the chief regions, pith, wood, and bark. Do you find concentric rings in the wood? How far apart are they and how many are there? These are the "annual rings," which indicate the number of periods of growth through which the stem has passed. Each "ring" is, of course, the end (cross-section) of a roughly cylindrical layer of tissue formed during a given period of growth. This

layer of tissue may be more accurately described as a truncate cone. Why?

2. Now study the cross-section under the microscope, and make a V-shaped drawing of a portion of the section, showing samples of each kind of tissue in proper position. What characteristics mark the end of one period of growth of xylem (wood) and the beginning of the next? State clearly the differences between the stem at this age and at the age when there is primary growth only. Outside of the wood zone there is the zone of "inner bark." What other term is applied to this tissue? Can you distinguish the cambium layer? Where is its exact location? How do the cells immediately on each side of it differ from the cambium cells? Notice particularly the medullary rays. How numerous are they? Remembering the special properties of cambium, what explanation can you give for the large amount of wood, the complete layer of phloem, and the numerous medullary rays? Do all the rays reach the pith? If not, how far do they reach, and how far do they extend toward the periphery? Are they alike in this respect? How do you account for this condition when compared with the distance the medullary rays extend in primary growth? Can you distinguish separate vascular bundles in this stem? What has caused the difference between this stem and the young *Aristolochia* stem in this respect? How many different tissues do you find in the bark region, and what characteristics does each show?

3. Compare a longitudinal section of this same stem with the cross-section just studied and distinguish each tissue. Notice especially the tracheæ. How

are they better constructed to give rapid transfer of water than the other parts of the stem? Note the **tracheids** (long cells with sharp ends). Are they parenchymatous or prosenchymatous? Why? Make a list of the various tissues of the stem and, opposite each, state what types of cells are found in each tissue.

4. Make a diagram of a stem 4 years old, clearly indicating the corresponding years' growth in phloem and in xylem, and showing some medullary rays for each year's growth. This should be a cross-sectional diagram, drawn not less than ten centimeters in diameter.

F. Comparison of Stem Structure:

Copy the following table into your note-books and indicate the presence of the characteristics by a cross under the proper heading, and opposite the name of the plant which shows the characteristic.

	Xylem	Phloem	Cambium	Bundles scattered	Bundles in a circle	Secondary growth	Bark	Medullary rays	Pith	Epidermis
Corn Stem										
Aristolochia less than one year old.										
Aristolochia 3–4 years old.										
Herbaceous Stem										

VIII. Mechanical Uses of Water in the Plant

A. Rigidity and Maintenance of Form:

Experiment 15.—To ascertain the cause of rigidity in beet tissue:

1. From a beet cut four slices about 5 mm. thick, 10 mm. wide, and 75 mm. long.
2. Place the slices as follows:
   (*a*) In tap-water.
   (*b*) In a 10 per cent. salt solution.
   (*c*) and (*d*) In boiling water for two or three minutes.

   Then place
   (*c*) In tap-water, and
   (*d*) In the 10 per cent. salt solution.
3. At the end of fifteen minutes observe and record the relative rigidity of the various slices, ascertained by carefully bending them.
4. Thoroughly rinse the slices, *b* and *d*, and then transfer them to tap-water. At the end of an hour (or sooner) observe them again and describe the result.
5. Explain your observations on the basis of your previous experiments.
6. What is one mechanical use of water in a plant tissue, and how is this accomplished?

Experiment 16.—To demonstrate longitudinal tissue-tension.

7. Obtain a petiole of rhubarb, or burdock, or a stalk of celery. With a scalpel make a lengthwise

cut for a distance of about 25 mm. from the end, and just beneath the surface.

8. Describe the position assumed by the severed piece. Illustrate by a diagram, natural size.
9. From another petiole cut off a portion at least 15 cm. long, with the cut surfaces normal to the edges. Record the *exact* length of the piece in millimeters.
10. With a scalpel carefully remove a thin strip of outer tissue along the entire length of the piece (or remove a strip of "bark" from a very young woody stem). At once try to replace it. Has it altered in length? If so, describe. Make another similar observation at the end of ten or fifteen minutes. What would you have to do to the strip to make it resume its former length?
11. Carefully measure the length of the excised strip about fifteen minutes after its removal. Record this measure, and calculate *the percentage of change* in length.
12. From another portion of the petiole cut off two strips from opposite sides (or the bark from a portion of some young woody stem). Place one of the excised strips in water, another in a 10 per cent. salt solution.
13. At the end of five or ten minutes compare the lengths of the two strips, (*a*) with each other, (*b*) with the portion of the stem from which they were cut. Explain what you observe.
14. From the preceding studies describe the condition of the tissues in a plant stem. To what is this condition due?
15. Of what advantage do you think this condition would be to the plant?

Experiment 17.—To demonstrate transverse tissue-tension.

16. Take short portions (about 15 or 20 mm. long) of some woody stem 15 to 20 mm. in diameter, and with the scalpel make a clean cut lengthwise through the bark, and remove the bark, being careful not to crack or break it.
17. At once, or at the end of four or five minutes, try to replace the bark. Describe your success in so doing. Draw, end view and side view.
18. What must be done to the bark in order to restore its original length?
19. From this study what further do you know of the condition of the tissues in a plant stem? Explain.

IX. Nutrition

A. The nutrition of plants is very similar to that of animals, with the exception that *all green plants manufacture their food out of inorganic chemical compounds*. Animals cannot do this. They must consequently receive their food ready-made. But there are some lower organisms (doubtfully animals) that possess the ability to elaborate their food out of inorganic compounds, while on the other hand, certain plants, such, for example, as the mushrooms and other plants wanting chlorophyll, lack this power.

B. The manufacture of carbohydrates is, in many respects, the most important function of green plants. Without it all life would be impossible, so that its study becomes of very great interest. We will first learn how to detect the presence of a carbohydrate such as starch, then study its occurrence in plants, and finally the process by which it is made out of simpler chemical compounds.

X. The Occurrence of Carbohydrates in Plants

A. The Test for Starch:

Experiment 18.—To ascertain the test for the presence of starch.

1. Place a bit of corn starch, about the size of a small shot, into a test-tube one-fourth full of water. Shake it thoroughly. Is starch soluble in cold water? Give a reason for your answer.
2. Bring the starch mixture to a boil over the flame of an alcohol lamp, or Bunsen burner. Describe the result. Is starch soluble in hot water? Give a reason for your answer.
3. Set this test-tube aside to cool for a moment or two.
4. Into a test-tube one-fourth full of clear water place 3 or 4 drops of iodine solution, using a pipette. Shake the mixture and describe the color.
5. Now place 1 or 2 drops of the iodine into the cooled, boiled starch mixture. Shake the mixture and describe the resulting color.
6. Pour one-half of this mixture into another test-tube one-half full of water. What color appears?
7. Describe a test for the presence of starch. (Note: The iodine is not the *test;* it is only the reagent used.)

Experiment 19.—To see if there is starch in (*a*) seeds; (*b*) stems; (*c*) roots.

8. Boil in water, in a test-tube portions of the above-mentioned parts of plants, and proceed with the starch test, as above outlined. Record the experiment as usual. Be careful to distinguish between your observations and your inferences.

Experiment 20.—Microchemical tests for starch.

9. If time permits of individual tests by the student, microchemical tests may be made by mounting in water, on microscopic slides, small portions of, first, commercial starch; second, material scraped from any soaked seeds (*e.g.*, corn, bean), a potato-tuber (a stem), any convenient fleshy root, in each case observing (and drawing) the shape, surface-markings, and characteristic groupings of the starch grains, then running under the cover-glass a drop of iodine solution, and observing the color reaction.

Experiment 21.—To see if there is starch in leaves that have been in sunlight.

10. Extract the chlorophyll from leaves of nasturtium, bean seedling, or other convenient large-leaved plant growing in sunlight, by placing the leaf first in hot water to facilitate the extraction; second, in hot alcohol, or, after they have been dipped in hot water, the leaves may be left in cold 80 per cent. alcohol until the following class period.
11. Describe the effect of the alcohol on the color of the leaf, and state your inferences as to the solubility or chlorophyll.
12. Place the leaf in a watch-glass, and irrigate it with iodine solution. After a few moments pour off the iodine, and observe the color of the leaf. This last observation is often made more striking by placing the leaf on a small piece of glass, and holding it to the light. State your inferences from this observation.
13. If preferred, de-chlorophyllized leaves may be cut into small pieces, boiled in water in a test-tube over a Bunsen flame, and the water then tested for the presence of starch.

B. Test for Sugar:

1. We have seen that starch is a practically insoluble carbohydrate. We also know that sugar is a readily soluble carbohydrate. The chemical formula for a molecule of starch is $C_6H_{10}O_5$. If we combine with this molecule one molecule of water (H_2O) we have a molecule whose composition is represented by the formula $C_6H_{12}O_6$ [$(C_6H_{10}O_5)_n + H_2O = C_6H_{12}O_6$]. This is *grape sugar.* Sugar, then, differs from starch in possessing relatively more hydrogen and oxygen in its molecule. The process of converting starch into sugar is termed **hydrolysis,** and since it converts an insoluble substance into a soluble one, it is a kind of **digestion.**
2. The sugar ordinarily used for culinary purposes is *cane sugar.* Its formula is $C_{12}H_{22}O_{11}$. Explain how cane sugar differs from starch chemically.

Experiment 22.—To demonstrate a test for the presence of grape sugar ($C_6H_{12}O_6$).

3. The reagent commonly used for this test is called Fehling's solution, from the name of the scientist who first employed it. The solution is prepared by mixing one volume of each of the following stock solutions with two volumes of distilled water (*e.g.*, 10 c.c. of each, and 20 c.c. of distilled water).
   (1) 17.5 grams of copper sulphate dissolved in 500 c.c. of distilled water.
   (2) 86.5 grams of sodium-potassium-tartrate (Rochelle salts) in 500 c.c. of distilled water.
   (3) 60 grams of sodium hydrate in 500 c.c. of distilled water.

   The mixture, properly made, has a clear blue color. If the Fehling's solution is not freshly

prepared, it should be tested, before using, by heating a portion in a test-tube until it boils. If a precipitate of red copper oxide does not form, the solution is good. It is better to make this test even with fresh solution.

4. Place a very small amount of grape sugar into a test-tube one-third full of water.
5. Shake the solution and gently warm it, then add a few drops of Fehling's solution.
6. Describe what results. The effect is due to the grape sugar reducing (*i.e.*, taking oxygen from) the cupric sulphate in Fehling's solution, forming cuprous oxide.
7. State the test for grape sugar.

Experiment 23.—To demonstrate a test for the presence of cane sugar.

8. Proceed as in the preceding experiment, using cane sugar instead of grape sugar. Observe and describe the result.
9. Prepare a second test-tube with a solution of cane sugar.
10. Add 2–3 drops of 50 per cent. hydrochloric acid, and boil the mixture.
11. Now add several drops of Fehling's solution (enough to neutralize the acid).
12. State the test for cane sugar.

Experiment 24.—To demonstrate the occurrence of sugar in plant tissues.

13. Test portions of onion, beet, sweet corn, sweet potato, etc., for sugar. Describe the result in each case.
14. Write a brief summary of what you have learned concerning the *occurrence* and *distribution* of carbohydrates in plants.

C. Tests for Cellulose:

1. Mount a few threads of cotton (cotton wool) in water under a cover glass. Cotton fibers are composed largely of cellulose. Irrigate under the cover glass with a little dilute sulphuric acid, and follow the acid with the iodine solution. Draw the various liquids under and away from the cover glass with a small piece of blotter or filter paper. Observe changes throughout under the microscope.

   Observe and record the color of the cotton fibers after treatment with iodine.
2. In a similar manner observe the color change when freshly mounted cotton fibers are irrigated with a solution of Chlor-iodide of zinc.
3. Mount thin sections of various portions of plant tissue, such as herbaceous stems, or bits of leaf epidermis, a few threads of *Spirogyra*, or plant hairs such as are readily obtained from leaves of mullein or squash, and apply the above tests. Of what substance are the untransformed cell-walls of plants composed?

XI. Formation of Carbohydrates

A. The Conditions Necessary for Carbohydrate Formation:

Experiment 25.—To ascertain if light is necessary for carbohydrate formation.

1. A green leaf, previously partly shaded by having a strip of black cloth closely affixed to both sides, is to be tested for starch as described under *Experiment* 21, after having been in the sunlight for several hours. Record as previously directed.*

Experiment 26.—Is chlorophyll necessary for carbohydrate formation?

2. As directed under *Experiment* 21, test a variegated leaf, having white areas devoid of chlorophyll. Make three drawings of the leaf, as follows: (*a*) showing (by shading) the distribution of chlorophyll in tissues; (*b*) showing the leaf decolorized; (*c*) showing (by shading) the areas that gave the starch reaction with iodine.

B. Effects of Light on Chlorophyll:

Experiment 27.—To show the need of sunlight for the formation of chlorophyll by chloroplasts.

1. Examine a seedling of any convenient plant that has been allowed to develop in darkness. Compare its color with that of another seedling of the same species grown in daylight.
2. Now place the seedling in diffuse sunlight for twenty-four to forty-eight hours. Record the result, and state your inferences.

* The "light screen," devised by Professor Ganong, for experiments in starch formation by leaves, is specially recommended for this experiment.

C. *The Exchange of Gases in Photosynthesis:*

Experiment 28.—To demonstrate the evolution of gas in photosynthesis.

1. Observe uninjured branches of *Elodea* growing in water in direct sunlight. (For individual experiments one or two branches in a large test-tube of tap-water will serve.) Describe what you observe, coming from the basal ends, or other parts of the stems.
2. Shade the plants for a moment by interposing a note-book or other convenient screen between them and the sun. Describe how the process just observed is affected.
3. Make a diagram of the apparatus and material, showing what you have observed.
4. Observe the bubbles among a mass of any green alga floating in water, and explain their presence.

Experiment 29.—To demonstrate what gas is given off in photosynthesis.

5. With a rubber band, or other convenient means, fasten together (not too tightly) the cut ends of 10 or 15 clean branches of *Elodea*, and place them into a glass funnel, with the cut ends extending upward. Invert the funnel into a jar of water. The surface of the water should rise an inch or two above the neck of the funnel.
6. Fill a test-tube with water and invert it over the neck of the funnel, being careful that no air enters the tube.
7. Place the apparatus in bright sunlight, and when sufficient gas has been collected in the test-tube, test it with a glowing splinter. How is the splinter affected by the gas? What gas does this test indicate? The best success of this experiment requires

that the gas be tested the same day that the experiment is set up. Especially *avoid setting up the experiment in the afternoon and testing the gas on the following morning.* Why?

Experiment 30.—To demonstrate what gas is taken into the plant in photosynthesis.

8. Into each of three large glass evaporating dishes, *A*, *B*, and *C*, place a glass bell-jar having a wide, open tubulature at the top. Into two of the bell-jars, *A* and *B*, place vigorous, green-leaved shoots. Into *C* place no shoot. Under each bell-jar place a piece of lighted candle, 2–3 in. high, supported on a flat cork. Now pour water into the evaporating dishes until it rises 2 or 3 in. up the side of the bell-jars. The burning of the candles shows that there is enough oxygen in the jars to support combustion.
9. Now cork the bell-jars air-tight with rubber stoppers. What soon results to the candle flames? What does this tell you of the amount of oxygen now in the jars?
10. Cover the jar *B*, containing a shoot, with opaque, black cloth, and set all three preparations in sunlight.
11. State, in a well-worded paragraph, the condition in each bell-jar as to light, chlorophyll, and the composition of the air.
12. At the end of two or three hours, carefully lower into each jar, successively, a lighted candle attached to the end of a long wire. Record your observation and inference for each jar, and your final inference as to what gas is taken into the plant in photosynthesis, and what conditions are necessary to the process.

XII. THE DIGESTION OF STARCH: TRANSLOCATION

A. The Starch-content of Leaves During the Day and at Night:[1]

Experiment 31.—To find out if starch is present in leaves gathered in darkness as well as in light.

1. Dechlorophyllized leaves of clover (or of one of the first five plants listed in the table in the foot-note below), collected (*a*) in bright sunlight, (*b*) several hours after sunset, will be tested by the instructor for the presence of starch.
2. Did both leaves probably contain starch during the day? From this experiment what do you know has taken place in the leaf gathered at night? Is starch soluble? What, then, must have occurred to the starch?

[1] MISS ECKERSON (*Bot. Gaz.*, **48**, 224–228. S 1909) recommends the following plants for this study, since in them phoosynthesis is very active, starch disappears from their leaves in darkness with comparative rapidity, chlorophyll is easily extracted, and the iodine reacts quickly:

Name of Plant	Disappearance of starch in darkness (T. 18°–22°C.)		Formation of starch in light (T. 20°–25°C.)		Iodine test
			Perceptible fig.	Good fig.	
	Nights	days	Minutes	Minutes	Minutes
Cucurbita Pepo	1	0	15	50	4–15
Helianthus annuus	1	0	30	120	5
Impatiens Sultani	1	0	30	120	5
Phaseolus vulgaris	1	0	20	90	5
Ricinus communis	1	0	20	60	5–15
Tropæolum majus	2	1	50	90	1
Zea Mays	3	2	30	120	5

3. Name two advantages to the plant of this new process you have studied.
4. *The changing of an insoluble substance to a soluble one and dissolving it is digestion.*
5. Briefly enumerate, in order, using the proper scientific terms, the processes that you have learned take place in a green leaf from sunrise to sunrise again.

B. Conversion of Starch to Sugar:

Experiment 32.—To see if starch may be digested to sugar by an enzyme.

1. Into a test-tube one-half full of a dilute starch mixture place several drops of iodine.
2. Add to this mixture a few drops of a solution of diastase.
3. At intervals of fifteen to twenty minutes test for sugar. Describe all color changes observed throughout the experiment.

C. State with special care and detail the inferences warranted by experiments 31 and 32.[1]

[1] NOTE· The study of proteins and fats is here omitted, not being considered essential in an introductory course.

XIII. Alcoholic Fermentation

A. The development of heat by alcoholic fermentation:

1. In these experiments fresh compressed yeast may be used, and, for a fermenting substance, either commercial molasses (20 c.c.) in water (100 c.c.), or Pasteur's solution, made up as follows:

Pasteur's Fermentation Solution

Grape sugar....................	150 c.c.
Ammonium tartrate...	10 c.c.
Magnesium sulphate...........	2 grams
Calcium phosphate.	2 grams
Potassium phosphate..	2 grams
Distilled water.................	838 c.c.

Experiment 33.—To ascertain what temperature change accompanies alcoholic fermentation.

2. Place about 5 grams of compressed yeast in 250 c.c. of the Pasteur's solution, shake well, and pour into a Dewar (pronounced, "Dū'er") flask.
3. Place a similar amount of distilled (or tap) water in a second flask.
4. Record the temperatures of both liquids at once, using two thermometers which should remain in the liquids until the experiment is over.[1] The experiment will work best if the liquids are at a temperature of about 25°C.

[1] The instructor will, of course, understand the necessity of carefully comparing the initial readings of the thermometers, where two or more are used, and of making necessary corrections in subsequent readings.

5. Set two Dewar flasks side by side where they will not be subject to great or unequal changes of external temperature.
6. At frequent intervals (*e.g.*, twenty minutes) during the next two hours, record the temperatures of the two fluids. Continue the records over as long a period as convenient, not exceeding twenty-four hours.
7. Tabulate the results, and form the figures construct two "curves," showing the rate and amount of temperature change in each flask.
8. State your inferences from this experiment.

B. The gaseous exchange in alcoholic fermentation:

Experiment 34.—To ascertain what gaseous exchange accompanies alcoholic fermentation.

1. Place 250 c.c. of fermenting mixture into a tall glass cylinder, and 250 c.c. of distilled water into a similar adjacent cylinder, as a control. At once test the air in the cylinders above the liquid with lime water, to see if the latter turns milky, as a result of the formation of a precipitate of carbonate of lime.[1]
2. After the test for CO_2, test the air in both cylinders with a lighted splinter or taper, to see if it contains sufficient oxygen to support combustion. The taper should be *rapidly* lowered into and removed from the cylinder. Why?

[1] If the members of the class are not familiar with the effect of CO_2 on lime water, this should be demonstrated by the instructor, using both chemically prepared CO_2 and the breath from the lungs, before proceeding with the experiments in fermentation and respiration.

The air in the cylinder may conveniently be tested by first dipping a small wire loop (*e.g.*, 10 mm. in diameter) into lime water. A film of the lime water will form across the loop, and may thus be transferred into the cylinder and out again.

4. Place a greased glass plate over each cylinder, or close the cylinders with a rubber stopper, and after an interval of about one hour (at a temperature of about 25°C.) repeat the tests for O and CO_2. Test again after two or more hours.
5. Record and interpret your results, especially discussing any circumstances that may have operated to affect the progress of the experiment either favorably or unfavorably.

C. *The Formation of Alcohol Demonstrated:*

Experiment 35.—To test for the presence of alcohol.

1. After the fermentation in the last experiment has proceeded for twenty-four hours, distill about 150 to 200 c.c. of the fermenting liquid, and redistill the first distillate.
2. Test a portion of the second distillate with a flame to see if it will burn. If it will, describe and explain the result.
3. The presence of alcohol may also be tested, in either the first or the second distillate, by adding to it several drops of a mixture composed of a strong aqueous solution of bichromate of potash, to which have been added a few drops of sulphuric acid. If a green color results, the presence of alcohol is indicated.
4. Briefly summarize the products of alcoholic fermentation, ascertained by the above experiments. From where did these products come, and what was the active agent in their formation?

XIV. Respiration

A. Anaerobic Respiration:

Experiment 36.—To illustrate anaerobic respiration.

1. Remove the seed-coats from three or four pea seeds that have soaked in water over night.
2. Fill a large glass test-tube with mercury, and invert it in a bath of mercury.
3. Place the pea seeds under the mouth of the inverted test-tube, and allow them to float to the top. Use every possible precaution to prevent air being carried up with the peas. Can the presence of air be entirely prevented?
4. Securely fasten the test-tube in the inverted position, with its mouth under the surface of the mercury in the bath, and during the next twenty-four to forty-eight hours observe the formation of gas, which replaces the mercury around the seeds.
5. Now introduce into the test-tube with the pea seeds a small piece of potassium hydroxide. If the gas given off by the seeds is CO_2 it will be absorbed by the potassium hydroxide, and the mercury will rise in the tube.
6. Do these seeds respire under *strictly* anaerobic conditions? Discuss, in your note-book, all the *pros* and *cons*, and endeavor to make a clear statement of just what this Experiment does and *does not* demonstrate.

B. Aerobic Respiration:

Experiment 37.—To demonstrate what exchange of gases accompanies the aerobic respiration of a living plant.

1. Place a vigorous potted plant on a ground-glass plate. By the side of it place a watch-glass full of lime water, or baryta water; over all place a glass bell-jar with a large tubulature at the top.
2. Make the joint between the bell-jar and the ground-glass plate air-tight by means of vaseline.
3. Test the air in the jar with a lighted taper to be sure that it contains enough oxygen to support combustion.
4. Insert a rubber stopper into the tubulature so as to make it air-tight, and set the plant aside, in a dark place. Why?
5. At the next laboratory period (preferably on the following day), and without disturbing the bell-jar, observe the color of the lime water in the watch-glass. What does it indicate?
6. Quickly and cautiously insert a lighted taper into the bell-jar through the tubulature. What results? What inference is justified?

Experiment 38.—To see if all parts of a plant, and non-green plants, respire.

7. Take six cylindrical glass jars, *a*, *b*, *c*, *d*, *e*, and *f*, provided with air-tight rubber stoppers.
8. Into (*a*) place a quantity of green leaves; into (*b*) green stems of some herb; into (*c*) young clean roots of some herb; into (*d*) freshly picked flowers; into (*e*) one or two fresh fleshy fungi; and into (*f*) nothing. Confine the plant material to one side of the jars by inserting a vertical partition of coarse wire netting.
9. Test the air in each jar to be sure that it will support combustion, then cork the jars air-tight, and place them in a convenient place.

10. At the next laboratory period carefully test the air in each jar with the burning taper. What inference may be drawn from the result?
11. Next, pour into each jar a bit of clear lime water, and wash the air by tipping the jars back and forth, *holding the half containing the plant material uppermost.* What conclusion does the result justify?
12. Clearly state the general conclusion from this experiment.

C. *The Temperature Change Accompanying Plant Respiration:*

Experiment 39.—To ascertain what temperature change accompanies the respiration of germinating seeds.

1. Place a quantity of germinating seeds (*e.g.*, oats, wheat, lupine) into a Dewar flask. Into a second Dewar flask place nothing.
2. Into each flask insert a thermometer (being sure first to compare their readings). The bulb of the thermometer in the flask containing the seeds should be well covered by them. Place the flasks where they will not be subject to great nor unequal changes of external temperature.
3. After twenty-four hours record the temperature indicated by each thermometer.
4. Thoughtfully discuss and interpret results observed.
5. Compare the process of fermentation with that of respiration. What inference is suggested by this comparison as to the real nature of respiration?

XV. The Influence of External Conditions on the Plant

A. The Influence of Gravity on the Direction of Growth:

Experiment 40.—To find how gravity affects the direction of growth of roots and shoots.

1. Choose two or three young seedlings of the pumpkin or lupine, with radicles about 10 mm. long.
2. Pin the seedlings horizontally on a cork and place in a moist chamber *in the dark* (why in the dark?) until the next period. A Petri dish will furnish a simple moist chamber.
3. Make a drawing of the seedlings in the horizontal position.
4. At the next laboratory period observe the position of both root and shoot. Draw.
5. Do the results give any evidence that the root *grew* downward and was not *pulled* down by gravity? Explain.

B. Influence of Light on the Rate of Growth of Stems:

1. Compare the lengths of the stems of seedlings *of the same age* that have grown, one in the dark, the other in the light. State the exact length of each of the stems in centimeters.
2. What do you infer is the effect of light on the rate of growth of stems *of the plants observed?*
3. Do you think this is true of all plants? (This point should be discussed with the instructor, in the light of more recent investigations on the subject. See especially, MacDougal, "*The Influence of Light and*

Darkness on Growth and Development." Memoirs of the New York Botanical Garden, No. II.)

C. *The Influence of Light on the Direction of Growth of Roots and Stems:*

Experiment 41.—To ascertain how one-sided illumination affects the direction of growth of roots and stems.

1. Fix a vigorous young seedling of white mustard with the root extending through the mesh of a piece of cheese-cloth stretched over the mouth of a large salt-mouthed bottle nearly filled with tap-water. The seedling should be as straight as possible, and stand vertically at the beginning of the experiment, with root extending well into the water.
2. Place the plant thus prepared into a box with a tightly fitting cover and a narrow, vertical slit at one side to admit the light. (A pasteboard shoe-box, stood on end, with the cover on, and the slit cut vertically in the cover will answer.)
3. Set the box and plant in a well-lighted window, with the slit toward the light.
4. Make a diagram of the entire apparatus and plant, in longitudinal section.
5. At the next laboratory period carefully remove the cover from the box and observe the position of the root and stem.
6. Draw another diagram similar to, and by the side of the first one, showing what you observe.
7. Compare the manner of response of the root and stem to one-sided illumination.

PART II

MORPHOLOGY AND LIFE HISTORY

I. Meaning of the Terms

A. Morphology.—Under Part I we considered various physiological processes, the primary result of which was to maintain the life of the individual plant. Most of those processes were found to be carried on by all plants. It is common knowledge, however, that plants differ widely from each other in both structure and habit of life. In other words, we recognize the fact of *variation.* This means that different plants solve the same problems of life in different ways. That phase of botany which concerns itself with a comparative study of structures, and seeks to interpret the *structural value* of an organ, no matter how it may be disguised, is termed the science of form, or **morphology.**

B. Life History.—Every plant, in the course of its existence, passes through a series of changes, in orderly sequence. Like an animal, every plant begins life as a single cell, the **egg,** or the equivalent of an egg; the egg (except in some of the lower plants) develops into an **embryo,** and the embryo grows and develops into an **adult.** The adult in turn, produces an egg, like the one from which it came, thus completing one **life cycle** and initiating another. These various changes constitute the life history of the individual.

C. Descent.—Just as one of the higher plants, such as a maple tree, begins life as a single cell, and becomes

more and more complex as it matures, so the plant kingdom as a whole, presents us with a series of organisms of gradually increasing complexity from the simplest one-celled forms to myriad-celled, complex forms. This fact suggests that the entire plant kingdom, like every individual plant, has had a developmental history, *the more complex organisms being derived from more simple ones by a series of gradual changes.* This is the **theory of descent,** or organic evolution. It teaches us that all organisms are related to each other, and is one explanation of why we so often find the same organ appearing again and again, under various guises, in plants externally unlike.

D. Classification.—The study of morphology and life histories enables us to recognize relationships among plants, and hence to build up a genealogical tree, showing lines of descent. Thus we can arrange plants, together with their nearest relatives, in groups; and related groups, again, in larger groups of successively higher orders. This gives us a rational basis for the classification of plants, and this phase of plant study is called **systematic botany,** for it makes possible the arrangement of plants in *a system, which endeavors to show how the plant kingdom, in all its diversity, has developed, or evolved.* This greatly simplifies our study of plants, for the number of different plants is too great for us to study every one; but if we recognize that each plant more or less imperfectly illustrates a group, then we can study an illustration of each group, and thus get a more nearly adequate picture of the kingdom of plants as a whole. The various systematic groups are given in *E* below.

E. An Outline of the Classification of Plants.[1]

[1] For reference, not memorizing.

The Great Groups of Plants

Division	Subdivision	Class	Order
I. Thallophyta	A. Algæ	1. Cyanophyceæ	
		2. Diatomaceæ	
		3. Chlorophyceæ	
		4. Phæophyceæ	
		5. Rhodophyceæ	
	B. Fungi	1. Myxomycetes	
		2. Schizomycetes (Bacteria)	
		3. Phycomycetes	
		4. Ascomycetes	
		5. Basidiomycetes	
		6. Fungi imperfecti (life histories imperfectly known)	
II. Bryophyta		1. Hepaticæ	Ricciales Marchantiales Jungermanniales Anthocerotales
		2. Musci	Andreales Sphagnales Bryales
III. Pteridophyta		1. Eusporangiatæ	Ophioglossales Marratiales Isoetales
		2. Leptosporangiatæ	Filicales Marsiliales
IV. Calamophyta		1. Sphenophyllineæ	Sphenophyllales
		2. Equisetineæ	Equisetales
		3. Calamarineæ	Calamarales
V. Lepidophyta		1. Lycopodineæ	Lycopodiales
		2. Lepidodendrineæ	Selaginellales Lepidodendrales
VI. Cycadophyta		1. Cycadofilcineæ	Cycadofilicales
		2. Cycadineæ	Cycadales
		3. Bennettitineæ	Bennettitales
		4. Cordaitineæ	Cordiatales Ginkgoales Gnetales
VII. Spermatophyta	A. Gymnospermæ	1. Pinoideæ	Coniferales Taxales
	B. Angiospermæ	1. Monocotyledoneæ	Pandanales Naiadales Graminales Arales Xyridales Liliales Scitaminales Orchidales
		2 Dicotyledoneæ	32 Orders, including
		(a) Archichlamydeæ Apetalæ Polypetalæ	Salicales Polygonales Ranunculales Rosales Violales Myrtales Umbellales
		(b) Metachlamydeæ Sympetalæ (= Gamopetalæ)	Ericales Polemoniales Plantaginales Rubiales Campanulales

II. Directions for Study

Polypodium vulgare (Common polypody)

A. Classification:

Division III. Pteridophyta (fern plants).
Class I. Leptosporangiatæ.
Order. Filicales.
Family. Polypodiaceæ.
Genus. *Polypodium.*
Species. *vulgare* L.

B. Habitat:

1. Record here your knowledge of the habitat of the specimen studied. The information is to be obtained from your own observation, and from your reading and class work.

The "Fern Plant"

C. Naked-eye Characters:

1. *General features.*
   (*a*) Note that the sporophyte is differentiated into **root** and **shoot.**
   (*b*) The leaf portion of the shoot is often called the **frond.** The fibrous roots and the leaves are borne on an underground stem **(rhizome).**
   (*c*) Make a sketch of the entire sporophyte (including only one leaf).
2. *The rhizome.*
   (*a*) Describe the natural attitude (*i.e.*, erect, or horizontal) of the rhizome. Where does it

grow? If it branches, describe its manner of branching.

(*b*) Does the rhizome bear any outgrowths besides the leaves and roots? If so, describe their structure, color, relative number, and location.

(*c*) The places on the rhizome where the leaves are borne are called **nodes.** What is the region between two nodes called?

Note.—The directions below (*d-i*) apply especially to the bracken fern, *Pteridium aquilinum.*

(*d*) Observe the end of a rhizome, cut squarely across. If preserved material is used, the cut surface should be kept moistened during the study. The observations may best be made from a piece 5 to 10 mm. thick, cut transversely and placed in a watch-glass of water. Do not cut or injure your specimen in any way, as it will be collected for further preservation at the end of the study.

(*e*) Distinguish the following tissue-regions:

(1) The **epidermis** (black in preserved material).

(2) Underneath the epidermis a narrow, dark-colored region of **hypodermal sclerenchyma.**

(3) Within the hypodermal sclerenchyma the fundamental tissue (**parenchyma**).

(4) Imbedded in the parenchyma two prominent elongate, dark-colored areas, the central sclerenchyma, or **stereome** (sometimes fused into one).

(5) Also imbedded in the parenchyma, and surrounding the inner sclerenchyma, several areas of **fibro-vascular bundles.** In fresh specimens these areas are yellowish, in preserved material they are lighter colored

than the inner sclerenchyma. How do they appear when a section is held to the light?

(*f*) Identify all the areas referred to above (*e*, 1–5) in a longitudinal section of the rhizome.

(*g*) At home, write a well-worded description of your observations under *e* and *f*.

(*h*) Make a diagram, 10 cm. in longest diameter, showing carefully the outline of the rhizome as seen in cross-section, and all the tissue-regions identified. Label each region, and underneath your drawing indicate the amount of enlargement.

(*i*) Underneath the first diagram make a second one, of the same enlargement, showing the relation of the tissues of the rhizome as seen in longitudinal section.

3. *The roots.*

(*a*) Describe the location, form, length, diameter, branching, relative number, and relation to each other (*i.e.*, close together or not; interwoven or not) of the roots. Draw.

4. *The leaves.*

(*a*) On what surface of the rhizome are the leaves borne?

(*b*) Note their differentiation into stem-like part, the **petiole,** and expanded portion, the **blade.**

(*c*) What is the color of the leaf? Describe and suggest a probable reason for any differences in color.

(*d*) Is the petiole **glabrous** (smooth, without hairs), or **pubescent** (hairy)?

(*e*) Is the blade **entire,** or divided into **pinnæ?** If the latter, do the clefts between the pinnæ

extend clear to the **midrib?** Compare the basal with the more distal clefts in this respect.

(*f*) Do the pinnæ appear to be all of the same age? If not, state reasons for considering some of them younger than others. Find evidence in your specimen of the method of formation of the **pinnæ.** Are they opposite or alternate?

(*g*) Describe and compare the **venation** of the blade, and of the individual pinnæ. Describe any constant relationship between the venation and manner of branching of the blade.

(*h*) Do the smaller veins **anastomose** (*i.e.*, have their ends united so as to form a network), or are their ends free? Compare the fern leaf in this respect with the foliage-leaf of a seed-bearing plant.

(*i*) Describe the appearance of very young, unexpanded leaves or portions of leaves.

(*k*) On the ventral surface of some of the leaves find the brownish fruit-dots, or **sori** (sing. **sorus**). Describe their location. Do you find them on the midrib of the frond or on the individual pinnæ? Are they between the smaller veins or on them? If the latter, on what part of the vein? Is their position constant (*i.e.*, always the same)? Are they located at the margin of the frond or pinna, or back from the margin? Describe their shape.

(*l*) Do the sori occur on all the pinnæ of a leaf? On all the leaves? Compare several specimens with reference to this point.

(*m*) Observe, using hand lens if necessary, that the sorus is composed of a group of small organs (**sporangia**). What do sporangia produce?

(*n*) Is there a membranous expansion (**indusium**) covering the sporangia in your specimen? Examine fronds of the other species of fern displayed in the laboratory and record your observations on this point, stating the names of the species observed.

(*o*) Leaves bearing spores are **sporophylls.** Fern leaves that do not bear spores are vegetative leaves or **foliage-leaves.**

(*p*) Do some of the sporophylls also function as foliage-leaves?

(*q*) Examine specimens of other kinds of ferns exhibited in the laboratory and see if your answer to (*p*) is true of all ferns. Describe briefly any exceptions found, giving the name of the fern.

(*r*) Make drawings, natural size, illustrating all features of the frond not clearly shown in your first sketch.

D. Microscopic Characters:

1. *The rhizome.*

(*a*) Study prepared slides of cross-sections of the rhizome. (*Pteridium aquilinum* is suggested as specially satisfactory for this study, *a–g*.)

(*b*) With the low power survey the section and identify the various tissue-regions already distinguished.

(*c*) With the high power, study the epidermis, and describe how many cells it is in thickness, the variation in thickness of the cell-walls, the **middle lamella,** separating the adjacent cells, and the canals, or channels, extending from the cell-cavity outward through the cell-wall. Do these canals ever branch? Do they form a

network? Is there any connection between the cell-cavities of adjacent cells?

(*d*) In a similar manner examine the cellular structure of the hypodermal sclerenchyma.

(*e*) Make drawings illustrating the features observed in (*c*) and (*d*), showing four cells of the epidermis, and three or four of the underlying sclerenchyma-cells. The cells should not be less than 10 to 15 mm. in diameter.

(*f*) Make similar studies and drawings of the cells of the parenchyma.

(*g*) Study one of the smaller fibro-vascular bundles and distinguish, from the circumference toward the center:

(1) The outer bundle-sheath, or **endodermis.**

(2) Within the endodermis, and adjacent to it, a single layer of starch-bearing parenchymatous cells, the **phloem-sheath.**

(3) Thick-walled **sclerenchyma-fibers.**

(4) Larger, thin-walled cells, having their cell-walls perforated, the **sieve-tubes.**

(5) Associated with the cells mentioned in (2)–(4), parenchyma-cells (**phloem-parenchyma**), containing starch.

(6) The cells mentioned in (3)–(5) constitute the phloem-region, of the bundle, or **phloem.**

(7) Within the phloem is the xylem-region, or **xylem,** composed of

(8) Large, conspicuous **tracheids,** whose walls have ladder-like (**scalariform**) markings, as seen in longitudinal section. Each tracheid is formed from a single, elongate, tapering cell; the living matter, or proto-

plasm, has disappeared, leaving only the cell-walls, unevenly thickened. In some plants (*e.g.*, the pine) the thickenings form "bordered pits."

(9) Smaller **tracheids,** resembling those of the phloem.

(10) Thin-walled cells forming the wood-, or **xylem-parenchyma.**

(11) Since the tissues of the fibro-vascular bundles are arranged circularly about a common center, the bundle is called **a concentric bundle.**

(12) Compare the various bundles and see if they are all of similar structure.

(13) Make a careful drawing showing the structure of the bundle, including all points mentioned under (*g*), (1)–(10). This drawing should be at least 75 mm. in longest diameter.

2. *The pinna.*

(*a*) Under the low power examine a small portion of one of the pinnæ or pinnules not bearing a sorus, and note the presence or absence of outgrowths.

(*b*) Can you observe any veins too small to be seen with the naked eye? If so, describe their relation to each other.

(*c*) Mount a small bit of the lower epidermis, and describe (*a*) any outgrowths; (*b*) the **stomata** and **guard-cells,** stating the number, shape, and contents of the latter. Describe the relative number and distribution of the stomata.

(*d*) Compare the stomata of the fern with those of a seed-bearing plant.

(*e*) Make drawings showing all features shown under 2, (*b*)–(*d*).

(*f*) Describe a cross-section of a pinna as shown in a prepared slide. Draw. Compare its structure with that of a foliage-leaf of a higher plant.

E. Non-sexual Reproduction:

1. Describe the possibilities of vegetative propagation of the sporophyte.
2. With a needle remove several sporangia from a sorus, mount them in water and study under low power.
3. Observe the differentiation of the sporangium into a stalk (**pedicel**), and a **spore-case,** containing **spores.**[1] Note the walls of the spore-case, and the row of thickened cells, the **annulus.** Describe these cells. Note the special place for opening of the spore-case, through which the spores escape between the **lip-cells.**
4. Make a drawing of the sporangium, about 35 mm. in shortest diameter, showing a portion of the pedicel.
5. Study the shape and surface markings, if any, of a single spore. Account for the shape. Are they all of substantially the same size, *i.e.*, is *Polypodium* a **homosporous pteridophyte?**
6. Make a drawing of the spore 15 mm. in longest measure.
7. Run a drop of glycerine under the cover-glass and carefully watch for the snapping motion of the sporangia by which, in nature, the spores are expelled.

[1] While the spore is formed by non-sexual reproduction (*i.e.*, by cell-division), the student should make sure that he understands whether it belongs to the sexual or asexual generation. Its study is included at this point because it is part of the material now being studied.

8. Explain the advantage to the species of having the spores expelled. Why would it not be as well if they merely dropped out of the spore-case?
9. If suitable material is at hand, study stages in the germination of the spores, and draw.
10. To which of the alternating generations does the fern-plant belong? Why?
11. Into what does the spore develop?

The Prothallus

F. Habitat:

1. State the locations and conditions of growth of the **prothallus** (also called **prothallium**), (*a*) in artificial culture; (*b*) in nature.

G. Naked-eye Characters:

1. Describe the exact size (in millimeters), color, and shape of the prothallus.
2. Is it differentiated into a **dorsal** and a **ventral** surface? If so, how are the two surfaces distinguished?
3. Describe the location and character of the **rhizoids.**

H. Microscopic Characters:

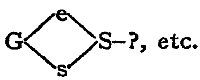
1. Mount a prothallus in water or clearing fluid, ventral side up, under a cover-glass.
2. Deşcribe the structure and contents of the cells.
3. Describe variations in thickness. Do you find a thicker central portion, or **cushion?**
4. Observe the **growing point** in the notch.
5. Study the location and character of the rhizoids. Are cross-walls present?

I. Sexual Reproduction:

1. Among the rhizoids find small elevations, the **antheridia.** Describe their number and distribution.
2. Nearer the notch observe the **archegonia,** appearing,

in cross-section, to be composed of four cells, surrounding an opening or canal.

3. Make a drawing, at least 5 cm. in longest diameter, showing all features of the prothallus thus far observed. By the side of this figure draw an outline of the prothallus, natural size.
4. In fresh specimens motile antherizoids or **sperms** may be found escaping from the antheridia and swimming in the water. If these are found, observe the body of the sperm and the **cilia.** How many cilia are there? Draw.
5. If prepared slides are supplied, study cross-sections of the prothallium passing through an antheridium and an archegonium. Describe accurately, noting the differentiation of the archegonium into a **neck,** containing a **neck-canal,** and a **venter,** containing an oösphere or **egg.**
6. Make a diagram of the section, of the same scale as the drawing in 3 above, and make *drawings* showing details of structure of the antheridia and archegonia as seen in longitudinal section.
7. To what class of reproductive bodies do the sperm and egg of the fern belong? To which of the alternating generations does the prothallus belong? Why? Why is it called a thallus?
8. Is this fern **monœcious** or **diœcious?** Explain.
9. What structure is the starting point of the sporophyte?
10. Diagram the life history of the fern for three generations, by continuing the following diagram; letting **G** = gametophyte; **s** = sperm; **e** = egg; **S** = sporophyte; **sp** = spore:



11. Make a diagram to show the life cycle of the fern, using arrows and words, arranged in a circle.

K. Nutrition and Growth:

1. Is photosynthesis carried on by both gametophyte and sporphyte? Transpiration? Absorption of water from the soil?
2. Explain the need of stomata in the sporophyte. Are they present in the gametophyte? Explain.
3. Discuss the presence or absence of a conducting system in the prothallium and sporophyte.
4. Explain how the presence of the cushion of the prothallium is related to the needs of the young sporophyte.
5. Is the gametophyte of *Polypodium* ever dependent upon the sporophyte for its nutrition? Its existence?

L. Comparison of Gametophyte and Sporophyte of Polypodium:

Copy the following table into your note-book, and mark *x* after the word gametophyte or sporophyte in the proper column.

TABLE I

Generation	Respiration	Photosynthesis	Stomata	Rhizoids	True roots	Capable of independent existence	Partly parasitic	Wholly parasitic	Bears sexual reproductive bodies	Bears asexual reproductive bodies	Has both vegetative and reproductive functions
Gametophyte											
Sporophyte											

Polytrichum commune (Common hair-cap moss)[1]

A. Classification:

Division II. Bryophyta (moss-plants).
Class II. Musci (mosses).
Order. Bryales.
Family. Polytrichaceæ.
Genus. *Polytrichum.*
Species. *commune L.*

B. Habitat:

1. *Polytrichum commune* is widely distributed, growing in the soil in fields and woods.

C. Naked-eye Characters:

The "Moss-plant"

1. Note that there are two kinds of leafy "moss-plants." The one having the cup-like tip is the male or antheridial plant; the other, without the cup-like tip, is the female, or archegonial plant. Compare the average height of the mature male and female plants. Do you find any outgrowth from the tip of any of the archegonial plants?
2. Are the moss-plants differentiated into root and shoot? Is the shoot further differentiated? If so, describe.
3. Briefly describe the extent and ramifications of the "root" system. Are these true roots, with root-hairs?

[1] With minor modifications the outline here given for the study of the moss will serve for species of *Mnium*, *Funaria*, or almost any other common moss.

4. Briefly describe the arrangement of the leaves on the stem (opposite, alternate, spiral). Are the leaves **sessile** or **petiolate?** **Simple** or **compound?** Is there a **midrib?** **Veins?** Compare the dorsal and ventral surfaces of the leaves. Describe any variations in the leaves on various parts of the stem. Describe the **margin** of the leaf-blade (*i.e.*, entire, notched, serrate, etc.), and the shape of its **apex** and **base.**
5. Compare the form of the leaves in the same regions of the male and female plants. Note especially the rosette of **perichætæ** (modified leaves) at the summit of the male plant. Compare them with the foliage-leaves below them.
6. Describe the form of the stem. Is it of uniform diameter? Does it branch? Compare the stems of the male and female plants.
7. Is the moss-plant a sporophyte or a gametophyte? Explain why.
8. Make suitable drawings, illustrating all points observed under *C* 1–6.

THE SPOROPHYTE

9. Select an archegonial plant with **sporogonium** attached. Distinguish the long stalk or **seta,** bearing at its summit the spore-case, or **sporangium.** How many millimeters long is the seta? Describe its surface; its diameter throughout; its shape in imaginary cross-section. If it is angled, how many angles are there? By taking hold of the seta near its attachment to the gametophyte and carefully pulling, separate the sporogonium from the gametophyte. State, with full reasons, whether or not the tissue of the **foot** appears to be

continuous with that of the gametophyte. Does anything like *grafting* of the sporogonium onto the gametophyte take place?

10. Do you find a swelling of the seta (**apophysis**), just beneath the sporangium? If so, describe and locate it accurately. Do its cells contain chlorophyll? Of what function is, or is not, the apophysis therefore capable?
11. Remove and study the cap (**calyptra**) that fits over the sporangium. Describe its shape, margin, character of surface, outgrowths, if any.
12. Study the color, shape, and other features of the sporangium disclosed by removing the calyptra. Measure its length and breadth. Describe its attitude on the seta (*i.e.*, erect, pendant, etc). Describe its outline in imaginary cross-section. If it is angled, record the number of angles.
13. Describe the shape and surface of the lid (**operculum**) at the end of the sporangium, and just under the calyptra.
14. Make a drawing, ten times natural size, showing the sporangium, the calyptra removed, and a portion of the seta.
15. Carefully remove the operculum and preserve it. On the margin of the sporangium, underneath the operculum, observe the circle of teeth-like organs (**peristome**). Record the number of teeth. Is this number constant? Is it always either even or odd? Draw.
16. In fresh dry specimens describe the effect of the breath upon the position of the teeth of the peristome.
17. Describe the membrane (**epiphragm**) within the peristome, and covering the end of the **capsule.**

Is it perforated? What is its relation to the teeth of the peristome?

18. Make a drawing, 30 mm. in diameter, illustrating an end view of the sporangium with the operculum removed. Make a drawing of the operculum, also 30 mm. in diameter.
19. With the razor carefully make a longitudinal section of the capsule, just to one side of its central axis. Observe the **wall** of the sporangium; a central organ (**columella**); and, between the two, a mass of **spores.**
20. Describe the structural relation of the columella to the epiphragm. What, in reality, is the latter?
21. Describe the relative number, color and attachment or non-attachment of the spores, so far as may be ascertained without the aid of the microscope. By what other name might we properly designate the sporogonium? Why?
22. Make a drawing, ten times natural size, illustrating everything observed under 18–20.
23. From the above observations construct a diagram of an *imaginary* cross-section of the sporangium near the middle. Compare the diagram with an actual cross-section.
24. Carefully preserve the sporophyte in a covered watch-glass or other convenient moist (not wet) place until the next laboratory period, or proceed at once with the following observations (*D*):

D. Microscopic Characters:

The Sporophyte

1. With a sharp scalpel remove a thin piece from the base of the sporangium, cutting parallel to the sur-

face, and mount it in water with the outer surface uppermost.

2. Examine the mounted tissue under the low, then under the high power, to see if stomata are present. If they are, describe them and their distribution. Compare them with the stomata of a foliage-leaf of one of the higher plants, including the number, shape, and other characters of the guard-cells. In like manner compare them with the stomata of the fern. State, with reasons, which type of stomata you consider the more primitive. Look for stomata on the surface of the apophysis.
3. Study thin cross-sections of the sporangium (sections of the half (*C*, 18) will serve). Identify the parts already studied, and their characters and relationship as seen in cross-section. Make your drawings at least 20 mm. in radius.
4. Describe the shape of the spores, and their manner of attachment or non-attachment, as seen under high power. Of how many cells is one spore composed? Make a drawing of three spores making each 10 mm. in longest measure.
5. Mount thin cross-sections of the seta, and study under high power.
6. Distinguish the outer layer, **epidermis.** How many cells thick is it? Observe the **central strand,** and between this and the epidermis a thin-walled tissue (**parenchyma**), and a layer of thicker walled cells (**sclerenchyma**). State how these various tissues may be distinguished from each other. Of what value is the sclerenchyma? The central strand is comparable with the fibro-vascular bundle of the seed-bearing plants. Draw.

The Gametophyte

The Leaf.

7. Remove an entire leaf and mount it in water. Observe under low, then under high power.
8. How many cells thick is it? Is it of uniform thickness? Describe. Are stomata present? Why? How is the midrib distinguished? Describe the leaf-margin and apex. Describe any differences in the two sides of the leaf.
9. Describe fully the contents of a single cell, as observed under high power.
10. Illustrate by suitable drawings all features observed under *D*, 7–9.

The Stem.

11. Study, under the low power, cross-sections of the stem mounted in clearing fluid (or use prepared slides).
12. Describe the tissues observed, and their relation to each other. Compare the structure of the gametophyte-stem, as seen in cross-section, with that of the sporophyte-stem, and name the tissues of the former, using the terms given above (*D*, 6).

E. Non-sexual Reproduction:

1. In some mosses a second gametophyte often develops from the tip of an older plant. This is called **proliferation.** Frequently this may be repeated a number of times, forming a chain of plants, each younger one growing out of the apex of the next older one. Examine the material at hand, and, if such a condition is found, describe it, with drawing. What kind of reproduction is this?
2. Explain the advantage to the species of the elongation of the seta.

3. If stages in the germination of the spores are available, study this process. The structure immediately developed from the spore is the **protonema.** Describe its color. Is it simple or branched? Are cross-walls present?
4. At certain points on the protonema observe **buds.** These buds develop into either male or female gametophytes (gametophores).
5. Which generation of the moss-plant always develops from the spores? Compare this with the case in the fern.

F. Sexual Reproduction:

The antheridia

1. Take a male gametophyte and, with a dissecting needle, carefully remove some of the antheridia borne in the rosette at the summit of the plant. Mount them in water, and study them under the microscope.
2. Describe the shape and other structural features of the antheridia. What is their color? Compare them with the antheridia of the fern.

   Do you find **paraphyses** associated with the antheridia? If so, describe them, and state how they may be distinguished from the antheridia.
4. If prepared slides are available, study longitudinal sections through the tip of the male gametophyte, observing the mode of attachment of the antheridia.
5. With high power study the **sperms** (spermatozoids) within the antheridia.
6. In fresh specimens the sperms may be seen swimming about in the water. If motile sperms are present, endeavor to make out the number and character of their organs of locomotion (**cilia**). Do

the cilia precede or follow as the sperm moves forward? Do the motions of the sperm appear to be purposeful or not? Give reasons for your answer.

7. Make drawings showing all features observed under *F*, 1–6. The antheridia should be at least 25 mm. long; the body of the sperms 10 mm. long.

The archegonia

8. With the female gametophyte make studies as directed above (*F*, 1–4).
9. In the archegonium distinguish the **venter, neck,** and **neck-canal-cells.** Is the archegonium sessile or stalked?
10. If prepared slides are available, identify the **oösphere,** or egg, and, in mature stages, the **neck-canal.** How many cells thick is the **wall** of the archegonium? Is this uniform? What has become of the neck-canal-cells?
11. Make a drawing, at least 35 mm. long, showing all features observed under *F*, 8–10.
12. Describe the conditions, processes, and organs involved in sexual reproduction in the moss. Explain whether or not it is of advantage to the moss-plants that they grow so close together.
13. Into what does the fertilized egg develop? Where does it develop?

G. Nutrition and Growth:[1]

The gametophyte

1. Is the gametophyte of the moss capable of elaborating its own carbohydrate food? Explain. Is it dependent upon the sporophyte at any period of its existence? Explain.

[1] This may be assigned for home work and serve as the basis of class discussion, or of written work to be handed in.

2. How does the possession of leaves affect the surface-area of the chlorophyll-bearing tissues? Explain how this affects the process of photosynthesis.
3. State the organs and processes by which water and inorganic salts are taken in by the gametophyte.
4. Explain the presence or absence of stomata in this plant.
5. By what organs is the respiration of the gametophyte carried on?
6. Does the gametophyte have to elaborate food in excess of its own needs? Explain. Explain the need or lack of need of conducting tissues in the gametophyte.
7. Name two ways in which the gametophyte is kept rigid and erect.

The sporophyte

8. Can the sporophyte lead an independent existence at any time in its history? Explain.
9. By what organ or organs, by what process, and from what source are water and dissolved food substances taken into the sporophyte?
10. Is photosynthesis possible in the sporophyte at any period of its existence? What is the source of its carbohydrate food?
11. Explain the need or lack of need of conducting tissues in the sporophyte. Compare the degree of development of these tissues in the sporophyte and gametophyte of the moss.
12. Explain the significance of the presence or absence of stomata in the sporophyte.
13. Refer to the question in *F*, 13, and explain the origin of the calyptra. To which generation does it belong? Explain.

14. Explain the advantage of sclerenchymatous tissue in the sporophyte. Describe the distribution of this tissue in the seta, and explain whether or not this is an added advantage.
15. After the sporophyte of *Polytrichum* begins to develop, does it grow continuously until maturity, or does a period of prolonged rest intervene? Is the same true with the sporophyte of the fern?
16. As directed in *I*, 10, p. 77, diagram the life history of the moss.
17. Outline the life cycle of the moss, as described in *I*, 11, p. 78.

H. *Comparisons:*

1. Write the following names of organs of the gametophyte of the moss in a column, and opposite them, in another column, the names of the corresponding organs of the fern; thallus, rhizoid, antheridiophore, archegoniophore, antheridia, sperm, archegonium, egg, paraphyses.
2. In a similar manner compare the organs of the sporophytes of the two plants, adding the names; stomata, foot, calyptra, columella, apophysis, sporangium.
3. In a third column make a list of organs of the moss not found in the fern; in a fourth column, the organs of a fern not found in the moss.
4. Compare the degree of organization of the gametophytes of the fern and the moss, as illustrated by *Polypodium* and *Polytrichum*.
5. In like manner compare the sporophytes of the two classes of plants.
6. State several reasons for regarding *Polytrichum* as either more or less highly organized than *Polypodium*.

Marchantia polymorpha (A Liverwort)

A. Classification:

Division II. Bryophyta (moss-plants).

Class I. Hepaticæ (liverworts).

Order. Marchantiales (marchantia-forms).

Family. Marchantiaceæ.

Genus. *Marchantia.*

Species. *polymorpha L.*

The Gametophyte

B. Habitat:

1. This plant grows very abundantly on the soil of flower pots and benches in nearly all greenhouses. In places it becomes a great annoyance to gardeners, and is very difficult to get rid of. Out of doors it grows in moist, shady places, frequently on rocky ledges by streams.

C. Naked-eye Characters:

1. Examine first a non-"fruiting" specimen.
2. Is the plant-body a thallus? Describe its color, outline, and manner of branching. What term is applied to this manner of branching? Does the plant possess **dorso-ventral differentiation?** If so, how are the dorsal and ventral surfaces distin guished?
3. Note the texture of the plant, to be ascertained by carefully breaking off a piece of fresh thallus.
4. Describe the appearance of the dorsal surface. The small areas into which it is marked off are **areolæ.**

Do you find evidence of an **air-pore** at the center of each areola? Is there a midrib?

5. The cup-shaped structures on the dorsal surface are called **cupules.** Do they occur on definite portions of the thallus (*i.e.*, margin, midrib, etc.), or irregularly? Describe their color, shape, height, diameter, margin. Are they sessile or stalked?
6. The non-sexual (vegetative) reproductive bodies within the cupules are brood-buds, or **gemmæ** (sing., **gemma**). Describe the color, shape and size of one (use hand lens). How are they attached to the plant? Do all the cupules contain them? Explain your observation on this point.
7. Examine the ventral surface of the plant. Describe its color and surface markings, and compare in these respects with the dorsal surface.
8. Note the root-like filaments or **rhizoids.** Describe their shape, color, dimensions, and distribution over the ventral surface.
9. Find purple, leaf-like structures (**scales**) among the rhizoids, and describe their form, position, and distribution.
10. Make careful drawings, showing:
    (*a*) The plant-body, natural size.
    (*b*) The surface markings of the dorsal surface, enlarged ten times.
    (*c*) A cupule, side view in perspective, enlarged ten times.
    (*d*) An outline of a gemma enlarged ten times.
11. Make a diagram of an imaginary cross-section of the plant-body, passing through one or more cupules (ten times natural size). Label all parts of the drawings.

D. Microscopic Characters:

1. The **rhizoids.**
   (*a*) With the forceps carefully remove a few of the rhizoids and mount them in clearing fluid. Examine them first under low, then under high power.
   (*b*) Do you find different kinds of rhizoids? If so, how are they distinguished? Are there cross-walls? Describe the contents of the rhizoids. Do they branch? Explain the shape of their tips, and the thickness of their cell-walls.
2. The **gemmæ.**
   (*a*) Remove several gemmæ with a scalpel, being careful not to cut or otherwise injure them, and mount them in a drop of water. Examine with low power.
   (*b*) Are the gemmæ more than one cell thick? Is there thickness uniform? Describe.
   (*c*) Find on the margin the **scar,** where the gemma was attached to its **pedicel,** or stalk.
   (*d*) Find two **vegetative notches,** 180° apart. How do they differ from the scar? Find papilla-like cells in these notches. Do they contain chlorophyll? Do they secrete mucilage? In the apex of each of these notches is a **vegetative point** from which a new thallus will develop. The mucilage protects it.
   (*e*) Are there any surface outgrowths? Is there dorso-ventral differentiation? Compare them in this respect with the thallus to which they give rise. So far as you can detect, would it make any difference which side up the gemma lay when it was sown?

(*f*) In the cells of a gemma do you find chloroplasts? A nucleus? **Oil drops?**

(*g*) Note the larger cells with clear contents from which the rhizoids will develop. Do they contain chlorophyll?

(*h*) Make a drawing 50 mm, in diameter, showing all the features observed under *D*, 2.

(*i*) Draw the outline of an imaginary cross-section passing through the center of a gemma.

3. The **thallus.**

(*a*) Under high power study the surface cells and air-pore. How many **guard-cells** are there? Compare the air-pores of *Marchantia* with the stomata of a foliage-leaf of a higher plant, and of the moss and fern. Are they true stomata?

(*b*) Study cross-sections of the plant mounted in clearing fluid.

(*c*) The careful study of the structure of the foliage-leaf, already made, makes it unnecessary to give detailed directions for these observations. Frame your own questions, to be answered by observing the mounted section. Note especially whether the tissues are differentiated, and, if so, compare with a foliage-leaf in this respect.

(*d*) Look for sections passing through air-pores, and compare their structure with that of the stomata of the leaf. What causes the surface appearance of the margins that delimit the areolæ?

(*e*) Describe the place and mode of origin of the rhizoids; of the cupules.

(*f*) Is the thallus of the same thickness throughout?

(*g*) Describe the chloroplasts. In some of the cells brown **oil globules** may be observed. If these are found, describe their location, and relative size. Do the cells that contain oil globules also contain protoplasm? Infer the source of the oil.

(*h*) Make drawings to illustrate all features observed under *D*, 3.

E. Vegetative Propagation:

1. There are two ways in which *Marchantia* can propagate itself without the intervention of gametes. In the first place, a portion of the thallus, broken off, is capable of developing into a mature individual. Somewhat, though not sharply, distinguished from this method is reproduction by means of the gemmæ. State two differences between a gemma and a fern or moss spore.
2. Incorporate the above facts into your notes at this point, using your own language, and state to what kind of reproduction each of the above methods belongs.

F. Sexual Reproduction:

1. Study plants having the upright stalks which bear the sexual reproductive organs.

The antheridial branch

Naked-eye Characters:

(*a*) The stalks having the mushroom-shaped tops bear the **antheridia,** and are hence called the antheridial branches, or **antheridiophores.** The expanded portion borne at the summit of the stalk, is the **antheridial receptacle.**

(*b*) Study and describe the stalks of the antheridiophores. On what region of the thallus are

these structures borne? On which surface do they originate? State their average height in millimeters. Describe the **grooves** on the surface. How many are there?

(*c*) Describe the color of the stalk. Are stomata present? Epidermal hairs or other growths? If so, describe.

(*d*) Do you find any antheridiophores that branch?

(*e*) Describe carefully the appearance of the upper surface of the antheridial receptacle, noting the occurrence and distribution of any structures or surface marks.

(*f*) Is this surface perfectly plane? If not, describe.

(*g*) Make drawings, twice natural size, showing all points observed under *F*, 1, (*a*)-(*f*), including a cross-sectional view of the stalk.

Microscopic Characters:

(*h*) Study and describe with drawings (5 cm. in diameter), the structure of the stalk of an antheridiophore as seen in cross-section.

(*i*) Using prepared slides, study thin longitudinal sections passing through a receptacle and portion of the stalk. Is there a differentiation into **epidermis** and other tissues? Describe in detail. Note the intercellular air-spaces. In what part of the structure do they occur? Suggest any advantage these air-spaces may be to the plant.

(*k*) Observe the chambers opening at the surface through necks, and containing the antheridia. How many antheridia in each chamber? Describe their shape, and mode of attachment. How many cells thick is the wall of the antheridium? Do the wall-cells contain chlorophyll?

(*l*) Describe variations in the size of the antheridia, and explain. Locate them according to size.

(*m*) Do you find papillæ (**paraphyses**) at the base of the antheridia? If so, of how many cells are they composed? Describe their shape and appearance.

(*n*) Describe the appearance of the contents of an antheridium. In the mature antheridium the contents are mature **antherozoids** or **sperms.** Younger antheridia contain **sperm-mother-cells.** Describe their appearance accurately.

(*o*) Illustrate by suitable drawings all the features observed under *F*, 1, (*i*)-(*n*).

The archegonial branch

Naked-eye Characters:

(*p*) The stalks having the umbrella-shaped tops bear the female reproductive organs or **archegonia,** and are called **archegoniophores.** The expanded portion at the top of the stalk is the **archegonial receptacle.**

(*q*) Do the archegonial and antheridial branches occur on the same plants? Measure the height of the stalks of several mature archegoniophores, and compare their average height with the average height of the stalk of the mature antheridiophore. Is the stalk of the archegoniophore grooved?

(*r*) Describe the markings of the upper surface of the receptacle, and compare it with the dorsal surface of the thallus. Are air-pores present? Record the number of rays on your specimen. Compare several specimens on this point.

(*s*) Describe the under surface. Note the fringed membranes (**perichætium**).

(*t*) Illustrate by drawings (×2) all structures observed under (*p*)-(*s*).

Microscopic Characters:

(*u*) Study and describe, with drawings (5 cm. in diameter), the structure of the stalk of an archegoniophore as seen in cross-section. Compare this with the antheridiophore (*F*, (*h*), p. 94).

(*v*) Using prepared slides, study longitudinal sections of the receptacle passing through one of its arms. If fresh or preserved material is at hand in sufficient quantity, the study may be made from material "teased out" on the slide.

(*w*) Study the tissues of the receptacle. Is there an epidermis? Stomata? Describe (*a*) the tissues just beneath the surface layer of cells; (*b*) those more deeply seated.

(*x*) Observe the flask-shaped **archegonia** hanging from the lower surface of the receptacle. Can you distinguish two regions—**venter** and **neck.** Note the passage, or **neck-canal,** leading from the venter through the neck, and opening at the summit. How many cells thick is the wall of the archegonium? Compare with the archegonia of mosses and ferns.

(*y*) Surrounding a mature archegonium observe the section of a cup-like structure, the **perigynium.**

(*z*) Within the venter of an archegonium just matured observe a single-celled **ovum,** or egg.

(*aa*) Make drawings illustrating all features shown under (*u*)-(*z*), and preserve the mounted section for subsequent study.

G. Physiology:

1. Is photosynthesis possible with the thallus? The antheridiophore? The archegoniophore? What correlation do you find between structure and function in this respect in the archegoniophore?
2. Explain the nutrition of the non-chlorophyll-bearing cells of the gemmæ. What is their nutritive relation to the gemma as a whole?
3. Is the gametophyte capable of an independent existence? Thoughtfully consider and then describe the correlation between structure and function in this respect.
4. In mature specimens grayish drops of liquid may often be found exuding on the dorsal surface of the antheridiophores. This liquid contains active antherizoids, or sperms. Mount some of it in water, and, under high power, observe the motion, organs of motion, and other structural features of these sperms.
5. When longitudinal sections of mature archegonia are mounted in water containing active sperms the behavior of the latter toward the former may be readily observed. If your material is suitable, make these studies.
6. How, only, can the sperms reach the egg? What external conditions would be favorable for this?
7. Of what advantage is it to the sporophyte to have the egg retained in the venter of the archegonium? Would this be of as great advantage in any aquatic plant as in a land plant? Why?
8. Is the small size of the sperms of any special advantage to the plant? Explain.
9. Explain any advantage in the greater height of the mature archegoniophore over that of the antheridiophore.

10. Enumerate several facts that insure a wide distribution of *Marchantia*.

The Sporophyte

A. Origin of the Sporophyte:

1. What is the process of the fusion of the egg and sperm called? What is the body that results from this fusion called? This body, by successive cell-division, develops into the sporogonium or sporophyte.

B. Naked-eye Characters:

1. In a mature specimen observe the small bell-shaped organs (**sporangia**), pendant on a stalk between the **perichætia.** The sporangia and stalk together form the **sporogonium,** or **sporophyte stage** of *Marchantia*. In fresh mature specimens an orange-colored mass containing spores is easily seen at the end of the **sporophyte.** Are the sporogonia borne on a line with the rays or between the rays?
2. Make drawings, four times natural size, showing the archegoniophore as seen from (*a*) the top; (*b*) the side; (*c*) the underside.
3. After making the drawings, as directed in *B*, 2, carefully dissect out one mature sporogonium and place it in a watch-glass to examine. Make a drawing 50 mm. long, showing all features observed, labeling the **foot, stalk,** and **sporangium.** Write a brief but clear description of the sporogonium.

C. Microscopic Characters:

1. If prepared slides are available of sections passing through the archegonia (*F* (*x*) above), find various stages in the development of the sporophyte within the archegonium. In nearly mature specimens

observe the attachment of the sporophyte to the receptacle by means of the **foot.** This study may be made to advantage with fresh or preserved material teased out on the slide. In such preparations there will be observed, surrounding the sporogonium, the membrane formed by the growth of the perigynium.

2. In the mount already made (or in a fresh mount of the orange-colored mass referred to in (*B*, 1, *p.* 98), observe the spore-mother-cells (**sporocytes**) or, in older specimens, the **spores** (in strands or separate, depending on the stage of development), and the elongate **elaters.** What is the size of the spores, and the number of cells of which they are composed? Describe their shape, and any surface marks observed. Describe any marks on the elaters. Of how many cells is an elater composed? Mount, *dry*, some of the mass that contains elaters, and observe, under the low power, their behavior as water is added. Draw.
3. Are the antheridiophores and archegoniophores sexual organs? Why? What are the sexual organs of *Marchantia?*
4. Name and classify (sexual or asexual) four different kinds of reproductive bodies produced by this plant. Consider carefully whether the spores, produced by the sporophyte, are sexual or asexual reproductive bodies.

D. Physiology:

1. Can photosynthesis take place in the sporophyte? Explain your answer.
2. From what source, by what organ or organs, and by what physical process does the sporophyte obtain its water and dissolved food? Compare it with the gametophyte in this respect.

E. Comparison of Gametophyte and Sporophyte:

1. Compare the degree of development or organization of sporophyte and gametophyte.
2. Copy the following table into your laboratory notebook and mark *x* after the word gametophyte or sporophyte in the proper column.

TABLE II

Generation	Respiration	Chlorophyll	Root-like organs	Capable of independent existence	Lives as a parasite	Produces gametes	Bears asexual spores	Functions, both vegetative and reproductive	Functions, chiefly reproductive
Gametophyte . .									
Sporophyte.									

3. State reasons why you consider *Marchantia* higher or lower in the scale of life than (*a*) the moss; (*b*) the fern.
4. Diagram the life cycle of *Marchantia*, as directed for the fern (*I*, 11, p. 78).
5. Indicate the life history of *Marchantia* for three generations, as directed in *I*, 10, p. 77.
6. Does the gametophyte ever produce another gametophyte directly? Does the sporophyte ever produce another sporophyte directly? If so, explain how. What phase intervenes between two gametophytes in the alternation of generations? Between two sporophytes? Is this always the case so far as your own studies show? Explain what is meant by the expression, "alternation of generations."

Fucus vesiculosus (Bladder wrack)

A. Classification:

Division I. Thallophyta.

Subdivision I. Algæ.

Class III. Phæophyceæ (Brown Algæ).

Order. Fucales.

Family. Fucaceæ.

Genus. *Fucus.*

Species. *vesiculosus L.*

B. Habitat:

1. Ascertain the habitat of this plant from your reading, class discussion, or field trip, and record it in your laboratory notes at this place.

C. Naked-eye Characters:

1. These characters may be best studied by floating a fresh specimen in a dish of sea-water. Material preserved in formalin should be rinsed under the tap, and then floated in fresh water.
2. Describe the color, shape, and size of the thallus. Does it form lateral branches or approximately equal terminal branches (**dichotomy,** forking).
3. Do you find any **holdfasts,** or organs of fixation? If so, describe them. State reasons why you think they are true roots or not.
4. Is there a **midrib?** A stalk, or **stipe?** Do you consider that the plant is differentiated into root, stem, and leaf? Give reasons.
5. Describe the distribution of **bladders.** Why is this species called "*vesiculosus?*"

6. Observe the swollen tips, **receptacles.** Do the tips of all the branches bear receptacles? How may they be distinguished from the bladders?
7. Carefuly note the dot-like projections on the receptacles. Find the circular openings in these projections, the **ostioles.**
8. Do you find ostioles elsewhere than on the receptacles? If so, describe their distribution over the surface of the thallus. Where are they not found?
9. Observe carefully the emarginate tips of the branches that do not bear receptacles. Do you find a groove in these tips? If so, is it in the plane of the thallus, or not?
10. Make careful drawings, natural size, showing all points noted under *C*.

D. Microscopic Characters:

1. Mount in water thin cross-sections taken through the thin expanded portion of the thallus, and study under the low power.
2. Note the differentiation of the tissue into central tissue or **medulla,** and a **cortical tissue.** How are the two distinguished?
3. Observe that the outer layer of cells of the cortical tissue is further differentiated into an **epidermoidal** tissue. Describe it. This outer layer is not a true epidermis, like the outer layer of cells of the leaf. In the younger portions of the thallus its cells, by division, give rise to the cells which form the underlying tissues. *None of the algæ possess a true epidermis.*
4. Is starch present in the cortical tissue? Chlorophyll? Note the layer of **cuticle** on the outer cell-walls of the epidermoidal layer.

5. Note that the cells in the medulla tend to form a thread-like network. Does starch occur in this tissue? Some of the cells unite, end to end, forming tubes to conduct liquids. Can you detect this?
6. Between the cells of both cortex and medulla is a mucilaginous layer, formed by the swelling and chemical transformation of the **middle lamella,** or layer that separates adjacent cell-walls.
7. Make a drawing showing the differentiation of tissues from the surface to the center of the thallus.
8. *Sterile Conceptacles:* Secure sections passing through one or more of the ostioles that do not occur on the receptacles. These ostioles will be found to open into spherical or pear-shaped cavities (**conceptacles**), imbedded in the cortical tissue. In viewing a cut end of the thallus with the naked eye, these cavities appear as minute dots underneath the epidermoidal layer.
9. Observe in these conceptacles, under the low power, numerous long hairs (**paraphyses**). Of how many cells is each composed? Do they extend through the ostioles to the surface? With what are they connected?

E. Physiology:

1. Of what advantage to an aquatic plant may the air-containing bladders be?
2. Does the plant grow attached to a substratum? If so, how?
3. How do you think the plant takes in its food elements?
4. Ascertain if the plant has chlorophyll. Is photosynthesis possible?
5. Would it be an advantage to this plant to have a system for conducting liquid nutrients from one

place to another? Is such a system present? In attempting to answer this last question recall the habitat of *Fucus*.

F. Vegetative Propagation:

1. Vegetative propagation is accomplished by the breaking off of branches which may float away and become established as new individuals.
2. Frequently, by a process of regeneration, dwarf branches are formed where portions of thallus have been torn away. Do you find instances of this in the material at hand?

G. Sexual Reproduction:

1. The sexual reproductive organs of *Fucus* are borne in fertile conceptacles, imbedded in the cortical tissue of the receptacles. In *Fucus vesiculosus* the conceptacles containing the female organs are on different plants, *i.e.*, the plants are **diœcious.** In other species they are both on the same plant, while in still other species (*e.g.*, *F. edentatus*) both kinds of organs are in the same conceptacle. In the two latter cases the plants are **monœcious.**
2. *The Male Conceptacles:*
   (*a*) Examine a longitudinal section of a **male conceptacle,** passing through the ostiole. Note the outline of the cavity. Describe its wall.
   (*b*) Observe the filaments (**paraphyses**) within the cavity, and describe the length, diameter, shape, and structure of one of them. Do any of these filaments project through the ostiole? Explain the feeling as a receptacle is taken between the thumb and fingers.
   (*c*) Are the filaments that pass through the ostiole similar to those that do not? On the latter observe the small ellipsoidal organs, **antheridia.**

Where and how are they attached? How many on each hair? Observe their contents, the **sperms** (antherozoids, spermatozoids).

(*d*) Make a drawing at least 50 mm. in longest diameter, illustrating all the above structures.

3. *The Female Conceptacles:*

(*a*) Study a longitudinal section passing through a **female conceptacle,** as directed above (G, 2). Compare them in all points with the male conceptacles.

(*b*) Observe the egg-bearing organs, **oögonia.** Describe their shape, size, color, place and mode of attachment, and number, and compare them in these respects with the antheridia.

(*c*) Describe the structure of the wall of the oögonium, noting especially whether it is composed of cells.

(*d*) Study the contents (**oöspheres,** or **eggs**) of the oögonium. How many are there?

(*e*) Make drawings showing all these points, as directed in *G*, 2(*d*).

4. *The Fertilization of the Egg:*

(*a*) Observe fresh plants that have been hanging in the air for about six hours, and see if you can observe an orange-colored fluid exuding from the ostioles of the male conceptacles. If so, mount some of this fluid in sea-water and examine it under the high power.

(*b*) Note the antheridia floating about, and the escaped sperms. Do the latter possess the power of locomotion? If so, how do they move? Describe their shape, relative size, and color.

(*c*) Make a drawing of three or four sperms, with the body about 10 mm. long.

(*d*) In a similar way, find the fluid exuding from the female conceptacles. What is its color? Mount a drop of it in sea-water and examine with the high power.

(*e*) Do you find any oögonia? Any free eggs? If so, how are the latter freed from the oögonia? Do they possess the power of locomotion? Compare their size with that of a sperm.

(*f*) Make a drawing (50 mm. in diameter) of an egg. By the side of the egg draw three sperms to the same scale, showing the relative size of egg and sperm.

(*g*) Prepare a mount containing both eggs and sperms, and endeavor, if possible, to follow the action of the sperms toward the egg, and the fusion of the two cells. With what act is fertilization completed?

(*h*) Do you consider *Fucus* a more highly or a more slowly organized plant than *Marchantia?* Give reasons for your answer.

Vaucheria sessilis (GREEN FELT)

A. Classification:

Division I. Thallophyta.

Subdivision I. Algæ.

Class II. Chlorophyceæ.

Order. Siphonales (Siphon-algæ).

Family. Vaucheriaceæ.

Genus. *Vaucheria.* (The only genus in the family.)

Species. *sessilis* (Vauch.) *DC.*

B. Habitat:

From your reading, class work, and material at hand, ascertain and record at this point in your notes the kind of localities where this plant occurs.

C. Naked-eye Characters:

Describe the color and "feel" of this plant, and the general form of the plant-body. What is the significance of the common name "green felt"?

D. Microscopic Characters:

1. Mount a portion of the material in water.
2. Is the plant branched? If so, is the branching lateral or **dichotomous** (*i.e.*, forked)?
3. Do you find cross-walls? Does the plant seem to be composed of cells? What is the outline of its cross-section?
4. Can you detect any signs of division into root and shoot? Do all portions of the filaments appear equally fresh and vigorous? Describe.

5. Do you find, on the end of any of the filaments, holdfasts? If so, describe them, and state their use to the plant.
6. Can you detect one or more nuclei? Any vacuole or vacuoles? Any individual **chromatophores** or chloroplasts? If so, what is their position and shape?
7. Describe the arrangement of the protoplasm within the filament.

E. Physiology:

1. Explain whether, or not, photosynthesis and respiration are possible with this plant.
2. Do you find any chromatophores dividing?
3. Do you find oil globules within the plant? Test dechlorophyllized plants with iodine for starch.
4. How are mineral matter and carbon taken into this plant? Explain the need or lack of need of special structures for conducting food and food elements from one part of the plant to another. Are such structures present?
5. Why is the plant not crushed by the weight of the water (when it grows in water), or by the cover-glass?
6. Can you detect any movement of the protoplasm? Observe carefully on this point.
7. Make careful drawings showing all features to which attention has been directed under *D*, and *E*, 2.

F. Asexual Reproduction:

1. Carefully examine the tips of numerous filaments and see if you find any of them slightly enlarged, and cut off from the rest of the filament by a cross-wall. Such a differentiated portion of the thallus of *Vaucheria* is a **sporangium;** its contents a **spore.**

2. If you are fortunate enough to have material at a suitable stage of development, you may, by careful observation, observe a spore escaping from the opening in the tip of the sporangium. If so, give careful attention to the mode of locomotion of the spore, and describe how its locomotion is accomplished. Since it has motion (as animals do) it is called a **zoöspore.** The zoöspore soon comes to rest.
3. If the material contains germinating zoöspores, carefully describe them.
4. Make drawings illustrating all you have observed under *F*.

G. Sexual Reproduction:

1. In "fruiting" material, observe the lateral organs that bear the gametes. These are the sexual reproductive organs. As is seen, they are of two kinds.
2. The larger, oval-shaped organ is called the **oögonium.** Is the oögonium cut off from the parent filament by a wall? On one side observe the **rostrum,** or beak, through which is an opening or pore. In material at a suitable stage may be observed a portion of the contents of the oögonium being voided or discarded. The protoplasm that remains in the oögonium now becomes organized into the larger gamete, or egg (oösphere). Is its wall composed of cells, or is it a unicellular organ?
3. By the side of the oögonium[1] find a slender branch, usually recurved at the end. Is this branch cut off from the parent filament by a wall? Is the tip cut off from the rest of the branch? This tip bears

[1] If the species is *V. geminata*, instead of *V. sessilis*, the reproductive organs will be found on the same lateral branch. The above directions will not apply in detail to any species except *V. sessilis*.

small gametes, that swim about by means of two lash-like cilia. They are the **spermatozoids,** or **sperms.** What is the organ that bears the sperms called? The base of the antheridial branch is the **pedicel,** or stalk.

4. Can you detect any sperms escaping? If so, observe and describe them carefully. See if you can find any empty antheridia.
5. Make careful drawings showing all points observed under *G*.
6. Is there a division of physiological labor in *Vaucheria?* Explain in detail.
7. Show, by a diagram, the life cycle of *Vaucheria*.
8. State the difference between conjugation and fertilization.
9. Draw an ideal diagram of a complete plant, showing all structures, and stages of their development.

Spirogyra sp. (Pond scum, Green silk)[1]

A. Classification:

Division I. Thallophyta.

Subdivision I. Algæ.

Class II. Chlorophyceæ.

Order. Conjugales.

Family. Zygnemaceæ.

Genus. *Spirogyra.*

Species. sp. (*i.e.*, not determined).

B. Habitat:

Ascertain from your own observations and from the text, and record at this point in your notes, the habitat of *Spirogyra*.

C. Physiology:

1. Explain, clearly but concisely, how the bodily form of this plant is maintained. Account for any variations in the shape of the cells.
2. Do you find any roots or other organs for anchoring the plant to the substratum? Do you think the plant is suitably organized for growing in running water? Explain. State a reason why roots are not necessary for this plant.
3. Explain the presence or absence of stomata. Do you find a cuticle?[2] Is photosynthesis possible with *Spirogyra?* Respiration? Explain.

[1] The morphological characters of this plant have already been studied (pp. 11–15). They should now be carefully reviewed, preparatory to the consideration of the physiology and reproduction of the plant.

[2] True cuticle does not occur, but a modification of the outer portion of the cell-wall, called the sheath, and which gives the plant its slippery "feel," is similar to cuticle, though not identical with it. This sheath is difficult to observe directly, though it may sometimes be identified on the outside of the filament at the places where the cross-walls occur.

4. Can you detect any difference between the cells, physiologically? From your own observations do you think there is any correlation between the structure of cells and their function? Explain clearly.
5. Is there any evidence in *Spirogyra* of a correlation between structure and environment? Explain.
6. A motion picture has recently been shown (by Prof. F. E. Lloyd) demonstrating the process of conjugation, and also the presence of **contractile vacuoles** (as in the animalcule, *Paramœcium*). Can you detect such organs?

D. Asexual Reproduction in Spirogyra:

1. From what you have already learned of *Spirogyra*, state the possibilities of vegetative propagation in this plant.

E. Sexual Reproduction in Spirogyra:

1. Use fresh material, if possible; otherwise preserved specimens, or prepared slides.
2. Observe the various stages in the fusion of two cells (gametes). Do the fusing gametes belong to the same, or to different filaments? Observe the **conjugation-tubes** connecting adjacent filaments.[1] What is their function? Their relative diameter? Try to find tubes in various stages of formation. Are their distal ends open before they come into contact? How is the opening made? Do the tubes grow together or merely touch each other?
3. Does conjugation seem to be a function of all the cells of the filament, or of certain cells only?

[1] The form of conjugation described in the outline above, is termed "scalariform" (ladder-like). Another type, known as "lateral" conjugation may frequently be met with, in which the gametes are formed by adjacent cells of the same filament. In less frequent cases the protoplast of a single cell organizes itself into a reproductive body (**aplanospore**) without conjugation. This process is a type of **parthenogenesis.**

4. Do the gametes pass from either filament to the other, or do the cells of a given filament all behave alike in this respect? In this connection see whether all the zygospores occur in one filament, or not.
5. Does the cell-wall of the **receiving cell** serve as the cell-wall of the zygospore, or does the latter form a new wall?
6. In a sentence define the term **supplying cell,** using the words gamete and conjugation.
   The passage of the protoplast from the cavity of the supplying cell to that of the receiving cell is accomplished by the action of the conotractile vacuoles.
7. If fresh material is studied, describe any observed differences in color between the mature zygospore and the non-conjugating cells; any structural differences between the cells of a supplying filament and those of a receiving filament. Do you observe any evidence of sexual differentiation in the filaments (*e.g.*, size-difference between filaments composed of supplying gametes and those composed of receiving gametes)?
8. Can you detect any constant structural difference between supplying and receiving cells in size, or in the size of the cell organs (*e.g.*, width of chlorophyll bands, diameter of pyrenoids, etc.)?
9. Explain whether *Spirogyra* represents a condition of isogamy or of heterogamy.
10. Make drawings of all the following features shown by your material, with each cell about 50 mm. long.
    (*a*) Two adjacent cells in which the conjugation-tubes are just beginning to develop.
    (*b*) Two adjacent cells in which the conjugation-tubes have just met.

(*c*) Two adjacent cells in which the active (supplying) gamete is passing through the conjugation-tube.

(*d*) Two adjacent cells in which the passage is complete.

(*e*) Two adjacent cells after conjugation is complete. Show carefully and accurately the details of structure of the zygospore.

11. Study stages in the germination of the zygospore as shown on the chart. State, in order, the processes that take place in the formation of the new plant (**mature zygote**) from the zygospore. Compare the plant of the new generation with its parents.
12. Is there a physiological division of labor in this plant? Explain in detail.
13. Draw a diagram showing the ancestors of a plant of *Spirogyra* for three generations.
14. To complete your notes on *Spirogyra*, write, at home, and before the next laboratory period, as clear and well-worded an account as you can of the life history of the plant.
15. Arrange ferns, algæ, mosses, liverworts, in a vertical column in the order of the complexity of their organization, placing the more highly organized near the top of the column. Write a clear statement of the reasons for your arrangement of the above classes.

Pleurococcus vulgaris (GREEN SLIME)

A. Classification:

Division I. Thallophyta.

Subdivision I. Algæ.

Class II. Chlorophyceæ (green algæ).

Order. Ulotrichales.

Family. Chætophoraceæ.

Genus. *Pleurococcus.*

Species. *vulgaris* Menegh.

B. Habitat:

1. From the material given you, infer where this plant grows. Leave a blank space in your note-book, and before the next laboratory period, record further observations on this point, made out of doors, noting especially the following points. Does the plant appear to be more abundant on one side of the object on which it grows than on another? Describe and explain. In general, what external conditions seem to favor its growth? Do you ever find it intimately associated with other plants? Describe.

C. Naked-eye Characters:

1. Describe the color of a colony of *Pleurococcus.* Can you distinguish the shape or other characters of an individual plant?

D. Microscopic Characters:

1. With the needle carefully scrape off a bit of the plant from a piece of moist bark or wood, and mount it in water.

2. Is the body of this plant differentiated into root, stem, and leaves? Is it composed of cells? If so, of how many? Make a thorough study of this point before you answer, and thoroughly consider how many cells you think are necessary in order to make one plant. State your opinion, with reasons. Compare the arrangement of the cells with those in *Spirogyra*.
3. Describe the color and shape of individual cells. Describe and account for any variations observed in the shape of the cells.
4. Carefully describe all the cell-organs you can identify in this specimen. Name all the cell-organs you cannot find. How does the chlorophyll occur in the cell of *Pleurococcus?* If you find chloroplasts state how many, their location, and relative size.
5. Make careful drawings showing all features so far as observed, with none of the cells less than 15 mm. in longest diameter.

E. Physiology:

1. How does *Pleurococcus* remain fixed to the substratum on which it grows? Are there special organs for this purpose?
2. Are there special organs for the taking in of nourishment from the substratum? How can the plant accomplish this process?
3. Is photosynthesis possible with *Pleurococcus?* Give reasons for your answer. Are stomata present? Why? Describe how CO_2 can be taken into the cell.
4. Are there any special organs of respiration? How can this process take place?
5. Do you think *Pleurococcus* is sensitive to stimuli from without? Give reasons for your answer.

F. Reproduction:

1. Do you find any cells that appear to be dividing? If so, carefully describe their appearance. What are the indications that a cell is dividing?
2. Do the cells tend to remain united after cell-division? Is this true of all of them? Describe.
3. Make three diagrams, showing (*a*) the life cycle of a *Pleurococcus* plant; (*b*) the descendants of one plant for six generations; (*c*) the ancestors of one plant for six generations.
4. Is there a division of physiological labor in this plant, or are all life functions performed by every cell?

Bacteria[1]

A. *Classification:*

Division I. Thallophyta.

Subdivision B. Fungi.

Class II. Schizomycetes.

Order. Bacteriales.

Family. Bacteriaceæ.

Genus. *Bacillus.*

Species (*e.g.*). *subtilis* (Ehrenb.) Cohn.

B. *Habitat:*

Bacteria are found almost everywhere except, possibly, far out at sea and upon the tops of lofty mountains. A very good source from which to take material for this exercise is the jar in which *Saprolegnia* was grown. If this is not now available use stagnant water from any source, or pickings from your teeth, using for this purpose a new toothpick.

C. *Microscopic Characters:*

1. Clean cover-glasses thoroughly and pass them through the flame of an alcohol lamp several times, taking care not to break them. If a hanging-drop preparation is not available a fairly satisfactory substitute may be made by cementing to a clean glass slide, with Canada balsam, two beheaded pins, laid horizontally, about twelve mm. apart.
2. With a sterilized needle put a large drop of water from the jar, thought to contain bacteria, on the clean cover-glass; invert the cover-glass and lay

[1] The directions for the study of bacteria were prepared by Prof. James S. Compton, Eureka College, Eureka, Illinois.

it upon the pins so the drop will be in the center of the glass. Place the slide on the stage of the microscope. Focus with great care, so as not to break the fragile cover-glass or wet the objective. The bacteria should be just visible with the low power; with the high power you should be able to make satisfactory studies.

3. Observe the bacteria in the mount. Note variations in shape: the spherical or **coccus** forms, the rods or **bacillus** forms, and, usually in such material, curved or **spirillum** forms. Note also the rapidity and character of the motion. Do they all move? Some have a vibratory motion; others move forward in a direct line.
4. The technique of staining is too difficult for the beginner in botany. The instructor will furnish stained and mounted bacteria for examination with the high-power. In some instances the instructor may use the oil immersion if it seems advisable. The following types are suggested for this study:
   Bacillus subtilis (hay bacillus).
   Bacillus proteus (decay), stained to show flagella.
   Bacillus tuberculosis.
   A *Streptococcus* and a *Spirillum*.
   In your notes enter data on (*a*) comparative size, (*b*) absorption of stain by bacteria, (*c*) grouping, (*d*) source.

D. Naked-eye Characters:

1. Prepare agar-agar culture medium, or use prepared medium supplied in a test-tube by the instructor. Melt the agar carefully over an alcohol or Bunsen flame, *beginning at the top*. (Why begin at the top?) When it is melted pour the agar into a sterile Petri dish, taking care to protect the dish (Why?) by

holding the lid directly over it, but far enough above to allow room for the test-tube. Any standard work on bacteriology will show how this is done, or the instructor may demonstrate for the class. After pouring replace the lid, and set the dish outside the window, or in the ice box, to cool. When cool and firm the dish may be infected and put away in a drawer or dark store room for a week.

2. Infect the agar-agar by removing the lid of the Petri dish and exposing the surface to the air of the laboratory, hall, library, or other place where people come and go. The exposure should be made for a definite time, uniform for all exposures, preferably three to five minutes.
3. When the bacteria have grown sufficiently to be visible in colonies (requiring usually a week or ten days, depending upon the temperature), bring out your cultures and examine them. Count the number of colonies visible to the naked eye, or with a hand lens, and, on the basis of one bacterium as the source of each colony, compute the number of bacteria falling per square centimeter per minute at the time of infection.
4. Observe carefully the differences in the colonies as regards (*a*) color, (*b*) elevation, (*c*) luster, (*d*) rapidity of growth.
5. Take two test-tubes of the same liquid culture-medium, preferably bouillon, and infect them in the same way. Boil one over a flame. Label both and set them away where they will not be disturbed. Observe at frequent intervals (*e.g.*, daily), for a week or ten days, noting especially turbidity and odor. If the instructor deems it advisable, some members of the class, instead of

boiling the contents of one tube after infection, may put into it ten drops of a 40 per cent. solution of formaldehyde.

E. Reproduction:

Do you find any bacterial cells that seem to be dividing? Is there a tendency for the cells to remain united after division? Read in some standard work on bacteria about the formation of spores.

Phycomyces nitens (or *Rhiozopus nigricans*)

A. Classification:

Division I. Thallophyta.
  Subdivision B. Fungi.
    Class III. Phycomycetes (alga-fungi).
      Order. Mucorales (the molds).
        Family. Mucoraceæ.
          Genus. *Phycomyces.*
            Species. *nitens* Kunze & Schmidt.

B. Habitat:

1. Upon what substratum is the *Phycomyces* growing? What atmospheric condition seems to be most favorable to its growth?

C. Naked-eye Characters:

1. Describe in detail the appearance of this plant as it grows. What is its color? Describe any variations in color.
2. Note the aerial filaments or **hyphæ.** Do they grow erect or horizontally? How many millimeters long are they?
3. On the ends of some of them observe the enlarged structure, the **sporangium.** Describe its shape. From its name, sporangium, what do you infer that it contains? Hyphæ that bear sporangia are **sporangiophores.** What does the term literally mean?
4. Compare the height of the sporangiophores bearing young (yellowish) sporangia, with that of those bearing more mature (dark-colored) sporangia. Explain the significance and advantage of this.
5. Using the hand lens, note the vegetative hyphæ that grow into the **substratum** (substance on which

the fungus grows), and over its surface. These filaments constitute the **mycelium.** Compare their diameter with that of the sporangiophores. Does their diameter appear to vary? If so, how? Do they appear to branch?

6. Make a drawing, illustrating all points observed. Make a diagram showing, in order, the relative heights of six sporangia of various ages. Indicate the scale used.

D. *Vegetative Propagation:*

1. If *Rhizopus nigricans* is used, study, with the naked eye or hand lens, the formation of **stolons** by this plant, and describe in full, with drawings, this process of propagation. This plant (*Rhizopus nigricans*) was at one time called *Mucor stolonifer.* Explain the appropriateness of this latter specific name. The generic name, *Rhizopus* (root-like foot), refers to the branching mycelial hyphæ, which form at the tips of the stolons. Explain the significance of the specific name *nigricans* (black).
2. How does *Phycomyces nitens* increase vegetatively?
3. Study and draw stages in the germination of spores that have been in sugar solution for twenty-four hours. (Use spores of *Phycomyces* or *Sporodinia*, as spores of *Rhizopus* do not germinate readily in sugar solution.)

E. *Microscopic Characters of the Mycelium:*

1. Mount in water a small portion of the substratum with the mold attached, and, if necessary, very carefully tease it out with the needles.
2. Study the mycelium. Is it branched? Are the mycelial hyphæ of the same diameter throughout? Are cross-walls present? If so, describe their frequency.

3. Make a drawing to illustrate the above points.

F. Physiology:

1. Describe the color of the sporangiophore and sporangium as seen under the microscope, and state whether this color is in the cell-wall or in the cell-contents.
2. If you detect any motion of the protoplasm (best seen in young sporangiophores) describe it accurately. Is it a true **circulation** (*i.e.*, in various directions in a closed circuit), a **rotation** (*i.e.*, up one side of the filament and down the other), or a **streaming** (*i.e.*, all currents apparently toward one and the same end of the filament). Suggest any advantage this motion would be in the nourishing of the plant; in the formation of sporangia.
3. Make a drawing of a portion of the hypha, at least 15 mm. wide, showing the appearance of the contents, and, with arrows, the direction of motion.
4. What foods does this fungus need? From where must they be obtained? Are they soluble? Can *Phycomyces* take in solid food? What process is necessary in our own bodies before we can utilize solid food? Must *Phycomyces* perform a like function? Is there a special organ for such a function? Must the process go on inside or outside of the body of the plant? Why?
5. Is photosynthesis possible with *Phycomyces?* Why? How must it get its carbohydrates?
6. Does *Phycomyces* respire? Give a reason for your answer.
7. What is the most obvious and important difference between the cells of *Phycomyces* and of *Spirogyra?*

G. Asexual Reproduction:

1. Study a sporangiophore. Is it of the same diameter throughout? Are cross-walls anywhere present? If so, describe their location.

2. Is the sporangium borne on the tip of the sporangiophore, or at one side? Are its contents separated from those of the sporangiophore? If so, how? Compare, on this point, young and old sporangia. Is there more than one sporangium on a sporangiophore? Within the wall of the sporangium observe the central **columella,** surrounded by the **spores.** Describe the shape of the columella. Are the spores numerous or few within one sporangium? Look for cases where the wall of the sporangium has ruptured, and the spores are mostly scattered, leaving the columella naked. Is the black pigment in the spores, or in the wall of the sporangium?
3. Illustrate by drawings all features observed under *G*, 1 and 2. Make the sporangium at least 20 mm. in diameter.
4. Describe the shape, relative size, color, and surface markings (if any) of the spores.

H. Sexual Reproduction:

NOTE.—For this study *Sporodinia* may be substituted, as it more readily yields suitable material.

1. Find conjugating branches. Describe their shape.
2. Find mature conjugating branches with the end contents cut off to form **gametes.** The remainder of the branch is now called a **suspensor.**
3. Find, on still more mature material, the gametes fused. What is the resulting structure called? Describe its appearance. If the material is suitable, describe the germination of this structure.
4. Illustrate, with a drawing, all features observed under *H*. Make the suspensors at least 25 mm. long, and other structures in proportion.

Saprolegnia (Water mold)

A. Classification:

Division I. Thallophyta.

Subdivision B. Fungi.

Class III. Phycomycetes.

Order. Saprolegniales.

Family. Saprolegniaceæ.

Genus. *Saprolegnia.*

Species. sp. (*i.e.*, not given).

B. Habitat:

1. The spores of this fungus are widely distributed, and develop readily under suitable conditions. Such conditions are commonly realized when a dead fly is placed in a dish of pond water, especially when some alga is added. The fungus will be sufficiently developed for study within five to seven days.

C. Naked-eye Characters:

1. Carefully observe the **hyphæ** as they grow, forming a halo about the body of the fly. What is the diameter of the halo? Its shape? Does its shape seem to be influenced by the shape of the fly's body? Do the filaments grow vertically upward and downward or only horizontally? What is the color of the halo?
2. Estimate the average length of the hyphæ.
3. Can you detect any evidences of the formation of **sporangia** at the tips of some of the hyphæ, and of sexual reproductive organs near the body of the fly? Use a hand lens if necessary.

4. Make a drawing, about 25 mm. in longest diameter, showing the appearance of this fungus as it grows on the body of the fly.

D. *Microscopic Characters:*

1. With the needle or scalpel carefully remove a few filaments and mount them in water. Examine with the low power.
2. Make careful comparisons of the tips of hyphæ enlarged to form sporangia with those not thus modified. Describe the appearance of the contents in each.
3. Do you find any cross-walls in the filaments?
4. Do you find vacuoles? Plastids? Nuclei?
5. Make a drawing showing the appearance of the tip of a vegetative filament (10 mm. in diameter).

E. *Nutrition and Growth:*

1. See if you can find hyphæ bearing empty **sporangia.** If so, do you find the hypha continuing its growth in length within the empty sporangium? Illustrate this point by a drawing.
2. Where do the vegetative hyphæ (**mycelium**) grow? Describe the nature of the surface of the fly's body. How can the delicate mycelia penetrate to the interior of the fly?
3. Upon what does this fungus feed? State in detail the necessary steps in the process of getting this food into the interior of the mycelium. In this connection make comparisons with *Phycomyces* (see *F*, 4, under *Phycomyces*, p. 124).
4. Is there any correlation here between the absence of chlorophyll and the habitat of the plant? If so, explain, and compare with *Phycomyces* and with *Spirogyra.*

F. Asexual Reproduction:

1. Carefully study again the terminal **sporangia.** How many times longer than broad are they? Compare the thickness of the sporangium walls with those of the remainder of the hyphæ. Can you detect any variations in the thickness of the sporangium walls? If so, describe and explain. Describe and account for the shape of the free tip of the sporangium.
2. Describe the shape of the zoöspores, or **swarm-spores;** their size; number in one sporangium; color. Are they all alike in these characters?
3. Endeavor to find swarm-spores escaping from a sporangium. Do they merely float away, or have they power of locomotion? Look for organs of locomotion? If you find them, describe their number, length, action, and general appearance. Do they precede or follow the zoöspore as it moves through the water?
4. Do the zoöspores ever move up or down through the water so as to be out of focus? If so, consider thoughtfully the thickness of the film of water in which they are, and try to form some conception of the size of a body that can move vertically beyond the range of vision in a film so thin. Briefly discuss this point.
5. Do you see any evidence that any two of these swarm-spores are in the process of fusion? Do their movements appear to be directed, or not?
6. Make suitable drawings to illustrate all points observed under *F.*

G. Sexual Reproduction:

1. Under suitable conditions female reproductive organs, **oögonia,** develop on certain hyphæ near the

body of the fly, and each oögonium develops a number of **eggs.** If oögonia are found, describe them carefully as directed above (*F*, 1) for sporangia, making suitable drawings. Do they always occur at the end of the hypha that bears them?

2. The male reproductive organs are **antheridial filaments,** growing either below the oögonia or on adjacent hyphæ.[1] They are of smaller diameter than the hyphæ. If you find these organs, carefully describe their appearance, contents, size, and relation to the oögonia. Illustrate all points observed with suitable drawings.

H. General Questions:

1. Do you find a physiological division of labor in *Saprolegnia?* If so, describe in detail.
2. State why you consider this plant higher or lower in the scale of life than *Phycomyces* or *Fucus*.
3. Describe all methods of dissemination of *Saprolegnia* that you can think of.

[1] The development of the egg-cell without fertilization (*i e.*, by *parthenogenesis*) is more usual than fertilization in *Saprolegnia*, so that fertilization, or even antheridial filaments, may be wanting.

Albugo candida[1] (Blister blight)

A. Classification:

Division I. Thallophyta.

Subdivision II. Fungi.

Class V. Phycomycetes.

Order. Peronosporales.

Family. Peronosporaceæ.

Genus. *Albugo.*

Species. *candida* (Pers.) Roussel.

B. Habitat:

This fungus is parasitic on plants belonging to the mustard family (Cruciferæ). It causes the "blister-blight," or "white rust," on the leaves and stems of the shepherd's purse (*Capsella bursa-pastoris*) and often on the radish.

C. Naked-eye Characters:

1. Describe the appearance (color, shape, size, etc.) of the blisters formed by this parasite on the **host-plant.** What organs of the host are affected?
2. Make drawings, natural size, showing all the features observed.

D. Microscopic Characters:

1. Study cross-sections of the host-plant taken through one of the blisters.
2. What causes the blisters? In what tissue or tissues of the host does the mycelium grow?

E. Nutrition and Growth:

1. In what form must carbon be supplied to this plant? Why?

[1] *Cystopus candidus* (Pers.) Lév.

2. In thin sections look for absorbing organs (**haustoria**), branching from the mycelium and penetrating through the cell-walls into the cells.[1] Describe their relative length, shape and general appearance. How far do they project into the cells? What do you infer is the function of these organs? Suggest a way in which they might be able to pierce the cell-wall. What other function must they perform besides the one you have already mentioned?
3. Where and how does this plant digest its food? What foods does it need? What is their source?
4. Is there any correlation between the absence of chlorophyll and the habitat of this plant? Explain, and compare with *Phycomyces* and *Marchantia.*
5. Make a drawing showing three cells of the host, with the adjacent mycelium and the penetrating haustoria.

F. Asexual Reproduction:

1. Observe the chains of spores (**conidia,** or conidiospores). On what are they borne? Describe their shape, color, size. Are they all of the same size? Which is the youngest conidium in a chain? Why do you think so? Of how many cells is each conidium composed? Are they attached to each other? If so, how?
2. Observe the conidia-bearing hyphæ (**conidiophores**). Describe their shape, and the appearance of their contents. Do they have cross-walls? Observe this last point carefully, and describe.
3. Describe in detail, from your own observations, the method of formation of the conidia.

[1] The haustoria are difficult to identify, especially with poor sections and too much time should not be spent in trying to detect them.

4. Make one drawing showing all points observed, including the tissues of both host and parasite.
5. Make a second drawing of two conidiophores, showing the attached chains of conidia and the mode of formation of the latter. In this drawing *make the conidia at least 5 mm. in diameter.*
6. Include in your notes at this point a brief description of the germination of the conidia. (The information should, if possible, be obtained from material supplied by the instructor, otherwise from lecture or reading.)

G. *Sexual Reproduction:*

1. The sexual reproduction of *Albugo* generally occurs in other parts of the host-plant, and later in the season than the asexual reproduction. The tissues of the host-plant containing the sexual organs of the parasite are generally enlarged (**hypertrophied**) and distorted.
2. In the material given you observe the large spherical **oögonium,** containing a single **oösphere** or egg surrounded by the so-called **periplasm** or epiplasm. Is the **oögonium** sessile or stalked?
3. Closely appressed to the oögonium at some point find the smaller **antheridium.** Describe its shape, and general appearance. Are its contents separated from those of the hypha by a cross-wall?
4. How does the male gamete (**sperm**) pass through the oögonium-wall and periplasm to the egg?
5. If your material is suitable, observe and describe the mature fertilized egg (**oösperm**). After fertilization the periplasm becomes transformed into the wall of the oösperm. Note the exospore (of one layer), and the endospore of three layers.

6. Make drawings showing all features observed under *G*.

H. General Questions:

1. Explain how *Albugo* is disseminated.
2. What weather conditions would favor its dissemination?
3. The blight caused by *Albugo* is difficult to eradicate. What characteristic of the plant helps to explain this fact?
4. Classify the phycomycetes you have studied as either **Zygomycetes** (Section 1), or **Oömycetes** (Section 2), and give a reason for your classification. Give the literal meaning of these two new terms.

Exoascus deformans (Peach leaf-curl)[1]

A. Calssification:

Division I. Thallophyta.

Subdivision B. Fungi.

Class IV. Ascomycetes (Sac Fungi).

Order. Protodiscales.

Family. Exoascaceæ.

Genus. *Exoascus.*

Species. *deformans* (Berk.) Fuckel.

B. Habitat:

1. What varieties of peach trees are most affected by this common and frequently destructive disease? Does it deform any other part of the plant than the leaves? How does it injure the tree?

C. Naked-eye Characters:

What is the character of the diseased tissues? What color are they? Draw and otherwise compare a healthy and a diseased leaf. Draw also a diseased twig, showing hypertrophied stem.

D. Microscopic Characters:

1. Make thin sections of diseased leaves or stems and examine. Draw some of the intercellular mycelium, as well as one or two of the asci.
2. How many spores in a mature ascus? Is there evidence of yeast-like budding of spores in the ascus? Are there any asexual spores formed? How is the infection spread?

[1] The directions for *Exoascus*, *Microsphaera*, and *Ustilago* (pp. 134–138) were prepared by Dr. E. W. Olive, formerly Curator in the Brooklyn Botanic Garden.

Microsphaera Alni (LILAC MILDEW)[1]

A. Classification:

Division I. Thallophyta.

Subdivision B. Fungi.

Class IV. Ascomycetes (Sac Fungi).

Order. Perisporiales.

Family. Erysiphaceæ.

Genus. *Microsphaera.*

Species. *Alni* (Wallr.) Wint.

B. Habitat:

1. Examine the specimens available, and record what kinds of trees and other plants are affected by this common fungous disease. What is the significance of the specific name *Alni?*

C. Naked-eye Characters:

1. What causes the dusty or "mildewed" appearance of the infected leaves? Are both sides of the leaf affected? Describe the distribution of the infection over the leaf-surface.
2. What are the minute yellowish or blackish bodies seen among the white threads? Make a drawing (natural size) to illustrate your observations.

D. Microscopic Characters:

1. Scrape off some of the whitish **mycelium** and examine it with low and high powers. Strip off the epidermis and try to find the sucker-like organs (**haustoria**) which grow down into the epidermis and absorb nourishment. Draw them, if found.

[1] See footnote, p. 134.

2. In younger material, study the **conidiospores,** arising from upright hyphæ (**conidiophores**). Draw. How are the conidiospores formed, and what is their function?
3. In older material, scrap off some of the black fruiting bodies (**perithecia**) and study. Draw one of the peculiar appendages. What is the funct'on of the appendages? Crush a perithecium and study the contained sacs (**asci**) and **ascospores.** How many asci in each perithecium? How many ascospores in each ascus? Draw to show these points.
4. What is the function of the ascospore? How are the perithecia formed; the ascospores?

Ustilago Zeæ (CORN SMUT)[1]

A. Classification:

Division I. Thallophyta.

Subdivision B. Fungi.

Class V. Basidiomycetes (Basidium fungi).

Order. Ustilaginales.

Family. Ustilaginaceæ.

Genus. *Ustilago.*

Species. *Zeæ* (Beckm.) Ung.

B. Habitat:

1. Upon what part or parts of the corn plant is the smut found? Does it differ in this respect from smut on wheat, oat, and other common grains? If so, how?

C. Naked-eye Characters:

1. Describe the appearance of the host as affected by the parasite. Cut into both young and old smutted areas and describe the appearance of the infected regions.
2. Draw, to show the part of the host-plant infected, and the degree of enlargement of the smutted tissues as compared with the normal.

D. Microscopic Characters:

1. With scalpel or forceps remove some of the spores from a smut-boil, mount, and study with both low and high powers. These are often called **chlamydospores** (from the Greek, *chlamy*, a mantle, and *spore*), because of their thick wall. They are not borne on a sporangiophore, but are formed in

[1] See footnote, p. 134.

chains by the fragmentation of the hyphæ, with accompanying morphological changes, including the thickening of the cell-wall.

2. Draw, to show the shape and surface markings of the spores.
3. If the spores are of the proper degree of maturity, they can be germinated on the surface of water, or on a hard manure-extract agar surface. If such viable spores are available, draw the germinating spore, showing the germ-tube, or **basidium (promycelium),** and attached **basidiospores (sporidia).** How many cells to the basidium, and how many basidiospores to each?
4. What is the function of the chlamydospore? Of the basidiospore?
5. From your reading, or otherwise, record how the corn-smut disease survives the winter, and how it is spread, as well as the amount of damage it may do.
6. Compare the corn smut with the wheat smut as to the character of the lesions, the method of infection, and the methods of control.

Agaricus campestris (MEADOW-MUSHROOM)[1]

A. Classification:

Division I. Thallophyta.
  Subdivision B. Fungi.
    Class V. Basidiomycetes.
      Series. Eubasidiomycetes (true or typical Basidiomycetes.)
        Sub-class. Hymenomycetes.
          Order. Agaricales.
            Family. Agaricaceæ.
              Genus. *Agaricus.*
                Species. *campestris L.*

B. Habitat:

1. From your own observation, and from the class discussion and assigned readings, describe the habitat of this plant.

C. Naked-eye Characters:

1. *Form.*—Describe the form of your specimen. If specimens of different ages are available, compare their forms, and describe any variations in specimens of various ages. Is the form of the mature specimens constant? Is their size constant?
2. *Color.*—Describe accurately, noting especially any variations in color.
3. *Structure.*—Note the differentiation of the plant-body (**thallus**) into an expanded portion (**pileus**),

[1] The outline for the study of a fleshy fungus has been prepared with special reference to the meadow mushroom (*Agaricus campestris*). It is general enough, however, with the exception of the outline of classification, to apply to any gill-bearing form. Indicate in your notes the exact genus and species given you for study.

borne on a stalk, or **stipe.** Is there a ring of tissue (**annulus**) around the upper part of the stipe?

(*a*) The **pileus.** Describe the shape, size, color, and any characteristic markings on its upper surface. Examine carefully and describe the margin of the pileus. Are there any characteristic elevations or depressions on the pileus? If so, state how many and where they are. Compare the color of the under surface in young and old specimens.

Describe the shape, arrangement, relative number and color of the lamellæ, or **gills.** Are the **margins** (free edges) of the gills entire or notched? Do they extend clear to the stipe? Are they attached to the latter? Do they all extend clear to the margin of the pileus? Describe any variations in size. Count the gills in a space of 10 mm., then calculate from the circumference of the pileus the total number of gills.

(*b*) The **stipe.** Describe its shape, and color, and any variations in color and diameter. Describe its mode of attachment to the pileus. Describe the method of attachment of the plant to the substratum. Is there a **mycelium** or other special means of fixation? If so, is it continuous with the tissues of the stipe?

(*c*) The **annulus.** If an annulus is present, describe its location on the stipe; its structure. Compare its structure with that of the membrane on the edge of the pileus. What is the relation between the membrane and the annulus in young specimens? When these structures are united they form a **veil.** Is a veil present

in young specimens examined by you? What do the annulus and marginal membrane represent?

(*d*) Make drawings, not less than life size, of both a young and a mature specimen, as seen from the side, labeling all parts.

(*e*) With a sharp scalpel or razor carefully divide your specimen longitudinally through the middle, and make a drawing illustrating all features shown in longitudinal sectional view.

(*f*) Make a third drawing showing the structure and outline of the stipe as seen in cross-section, and a fourth drawing, showing the outline of the gill as seen in cross-section.

D. Microscopic Characters:

1. When suitable material is available, note and describe the mycelium, extending through the soil.
2. The **annulus** and **stipe.** Mount (in clearing fluid or water) thin longitudinal sections passing through the stipe. Is the stipe composed of distinct tissues, *e.g.*, like the hypocotyl of *Ricinus*, or the thallus of *Fucus?* If so, describe. Observe that the stipe is composed of **hyphæ.** Of what is the annulus composed, and what is its relation to the tissue of the stipe? Do the hyphæ have cross-walls or **septa?** If so, what angle do the septa make with the walls of the hyphæ? Do the hyphæ branch?

   Do you find spaces between the hyphæ? If so, describe their size and distribution. Make a drawing to show the features observed under 2.
3. The **pileus.** Mount (in clearing fluid) a thin longitudinal section passing through the stipe, pileus, and a portion of a gill. Examine under low power. Can you trace the hyphæ of the stipe into the pileus?

If so, describe their arrangement within the pileus. Do they extend into the gills?

4. Make a diagram, life size, to illustrate the relation to each other of the hyphæ of the mycelium, stipe, annulus, pileus, and gills.

E. Reproduction:

1. Mount (in clearing fluid or water) thin cross-sections of a gill.
2. Observe the differentiation of the gill into a central tissue or **trama,** an outer, spore-bearing tissue, or hymenial layer (**hymenium**), and, between these two, a **sub-hymenial layer.** Of what are these tissues composed? How are they distinguished from each other? Show by diagram the position of these three layers.
3. The **hymenium.** Using a prepared slide, state the direction of its cells relative to the surface of the gill. Distinguish in it two kinds of cells, (*a*) club-shaped ones, **paraphyses,** (*b*) **basidia** (sing., *basidium*), bearing **sterigmata** (sing., *sterigma*). How many sterigmata terminate each basidium? Observe the spores, borne on the **basidia** and hence called **basidiospores.** Are the basidiospores all of the same size? Explain. Describe their color, shape, surface markings (if any), and the number of cells of which they are composed. Is the color of the spores constant? To what is the color of the gills due?
4. If a pileus with the stipe removed is placed with the gills down over a clean, smooth piece of paper (black or white according to the species used), then covered with a tumbler or other suitable glass dish, and left over night, a print of the spores, as they fell from the gills, will be found on the paper

in the morning. Study a **spore-print** of this species, describe it fully, and state how it was formed.

5. Make accurate drawings showing all features observed under *E*, 2 and 3.
6. Sexual reproduction is unknown among the Agaricales.
7. Diagram the life history of the species studied.

F. Nutrition and Growth:

1. Could the mushroom exist independently of other plants? Consider this question thoughtfully and answer as fully as possible in a well-worded paragraph.
2. Suggest an explanation for the rapid growth of mushrooms.

G. General Questions:

1. State whether there is a division of physiological labor in this plant, and, if so, to what extent.
2. In what ways may this plant become widely distributed?
3. State, with reasons, whether you consider the mushroom a more or less highly developed plant than (*a*) *Spirogyra;* (*b*) *Polypodium.*

Puccinia graminis (WHEAT RUST)

A. Classification:

Division I. Thallophyta.

Subdivision B. Fungi.

Class V. Basidiomycetes.

Series. Protobasidiomycetes (Preliminary group of the series).

Order. Uredinales.

Family. Uredinaceæ.

Genus. *Puccinia.*

Species. *graminis* Pers.

B. Habitat:

1. Examine the specimens exhibited in the laboratory, and state what plants are infested with this parasite. What is the significance of its specific name (*graminis*)?
2. *Puccinia graminis* requires two different kinds of hosts in order to complete its life history. One of these is the barberry (*Berberis*). The barberry-stage, however, is not absolutely essential, for in certain regions, *e.g.*, Australia, the Central Western States, and California, where the barberry does not naturally grow, this stage may be omitted, the fungus being perhaps carried over the winter season on winter wheat, or possibly by mycelium in grain, or even by urediniospores.

ÆCIAL STAGE (on *Berberis*)

C. Naked-eye Characters:

1. Study the infected leaf of the barberry. On its under surface observe the cluster-cups or **æcia** (sing., *æcium*).

2. On the upper surface observe the small dots, the **pycnia,** containing **pycniospores** (pycnidia[1]). What is their color? Their shape? What relation does their position bear to that of the æcia?
3. Study both æcia and pycnia with the aid of a hand lens. Describe carefully the appearance of the infected areas.
4. Make a drawing, life size, of the barberry leaf, showing the features under *C*, 1–3.

D. Microscopic Characters:

1. Study longitudinal sections through an æcium, using low power.
2. How are the infested tissues of the host affected by the parasite?
3. Note the **æciospores.** Describe their shape. Are they all of the same size and shape? How are they produced? What is the cause of the cluster-cups that appear on the leaf-surface?
4. Make out all you can of the details of the **mycelia,** and their relation to the cells of the host-plant, and describe.
5. Make a drawing of two æcia in different stages of development, one before the epidermis of the leaf has been ruptured. Make the æcium at least 30 mm. in longest dimension.
6. Make a study, similar to that outlined in *D*, 1–5, of the pycnia, as seen in longitudinal section. Observe the slender threads and the minute spermatia.
7. What is the function of the æciospore? Of the pycniospore?

[1] Probably not *spermagonia* bearing *spermatia* (male reproductive cells), as was formerly supposed. Cf. Christman, A. H , Bot. Gaz. 44: 93, 1907; and Gager, *General Botany*, under this head (Chapt. XXII).

UREDO-STAGE (on Wheat, *Triticum vulgare*)

E. Naked-eye Characters:

1. Study the diseased spots on the leaves of the wheat. Use the hand lens, if necessary, to make out the features clearly.
2. Is the shape of the spots (**sori,** sing., *sorus*) uniform and characteristic?
3. State their color.

F. Microscopic Characters:

1. Study longitudinal sections, through a **uredo-sorus.** If the material is not fresh, remove some of the contents of the sorus with a needle, and mount in water. Study under high power.
2. Describe the color, shape, and relative size of the cells. Are there any surface marks? Do you find any remnants of the **pedicel** to which the **urediniospore** ("summer spore") was attached? What can you say of the thickness of the cell-wall?
3. Of how many cells is the urediniospore composed?
4. Make careful drawings of two or three urediniospores, at least 15 mm. in longest measure.
5. State the function of the urediniospore.

TELIAL STAGE (on Wheat)

G. Naked-eye Characters:

1. Study the **telial sori,** as directed under *E*, above. Describe the order of their distribution.

H. Microscopic Characters:

1. Study as directed under *F*, above.
2. Include in your notes, at this point, a description of the germination of the **teliospore** (teleutospore). What is its function? State the function of the **basidium** (promycelium), and of the "spring spores," or **basidiospores** (sporidia).

I. General Questions:

1. What features seem to you to make this parasite easily distributed, and difficult to eradicate?
2. State, with reasons, whether you would consider *Puccinia graminis* higher or lower in the scale of life than *Vaucheria* (or *Fucus*), and *Albugo* (or *Mucor*).
3. Write a brief summary of the life history of *Puccinia graminis*, and devise a diagram to illustrate this.

Note

From the fern to the wheat rust we have studied plants in the descending order, from higher to lower in the scale.

We now return to the ferns, taking the quill-wort (*Isoetes*), illustrating the Eusporangiatæ.

The systematic relationship of the Isoetaceæ is doubtful. On the basis of certain structural features of the gametophyte (*e.g.*, the structure of the archegonia, and the possession of multiciliate sperms), some botanists class them with the Pteridophyta. On the other hand, some features of the anatomy of the sporophyte (*e.g.*, the possession of a *ligule* on the sporophyll) suggests that they are more closely related to *Selaginella* (Lepidophyta).

Isoetes (QUILLWORT)

A. Classification:

Division III. Pteridophyta.[1]

Class I. Eusporangiatæ.

Order. Isoetales.

Family. Isoetaceæ.

Genus. *Isoetes.*

Species. (*e.g.*) *lacustris L.*

B. Habitat:

Some forms grow on the bottom of ponds, others in moist meadows, or on the margins of bodies of water.

THE SPOROPHYTE

C. Naked-eye Characters:

1. *General Features.*
   (*a*) Note the differentiation of the plant into root and shoot, and of the shoot into stem and leaf.
   (*b*) Make a sketch, natural size, showing the general appearance of the entire plant.
2. *The Stem.*
   (*a*) Without removing any of the leaves or roots, ascertain all you can about the shape, size, branching, and other characters of the stem, and describe.
   (*b*) With a sharp scalpel, make a cross-section of the stem through the middle, being careful not to remove any of the leaves or roots.

[1] See note, p. 147.

(*c*) Describe the outline of the stem as seen in cross-section. Note the **longitudinal furrows** which give it a lobed appearance.

(*d*) Are the roots attached to any special region of the stem? If so, describe.

(*e*) Study the cross-section, identifying the central **(vascular) cylinder,** the **epidermal layer,** and, between the two, the fleshy tissue composed of several regions that are not distinguishable to the naked eye.

(*f*) Make a drawing (× 3) showing the outline of the stem in cross-section, the tissue-regions observed, and the attachment of the roots.

3. *The Roots.*

(*a*) Describe the general appearance of a single root. Does it taper? Note that it is slightly fleshy. Are the branches forked at the tip (**dichotomous**), or lateral? Dichotomous branching of roots is very rare.

(*b*) Make a drawing (× 2) showing these features.

4. *The Leaves.*

(*a*) Carefully remove one of the outer leaves at its point of attachment to the stem, first noting carefully which is its inner (**ventral**) surface, and which is its outer (**dorsal**) surface.

(*b*) State whether the leaf is **sessile** or **petiolate.** The end by which it is attached is the **leaf-base;** the remainder of the leaf is called the lamina or **blade.** Note that the blade is **subulate** (awl-shaped).

(*c*) Describe the exact length of the leaf in millimeters. Observe the slight shallow groove or flattening. On which side of the leaf is it?

(*d*) Hold the leaf up to the light and observe the single vascular bundle, surrounded by **air chambers** separated into compartments by numerous **diaphragms.**

(*e*) Make a drawing (× 2) illustrating the features mentioned in 4, (*c*) and (*d*).

(*f*) Make a cross-section of the blade near the middle, and note the number of air chambers surrounding the vascular bundle.

(*g*) Make a diagram, 10 mm. in diameter, showing the leaf-structure in cross-section.

(*h*) On plants which grow under water no stomata occur, but they are present on leaves that grow exposed to the air. Are stomata present in your specimen? If so, make a drawing of two or three, each 15 mm. in longest diameter.

(*i*) Study carefully the expanded base of this leaf, noting the membranous margins.

(*k*) Directly above the **leaf-insertion,** and on the ventral (inner) surface, observe the cavity or pit (**fovea**)**,** containing the single **sporangium.**

(*l*) Note the thin membrane (**velum**) extending over the sporangium. The velum is formed by the projection of the margin of the fovea. It is absent in some species, and in *I. lacustris* it does not completely cover the sporangium. In terrestrial species there is no opening through it. State the shape and location of this opening in your specimen, using a hand lens for the observation.

(*m*) Above the fovea find a flat, membranous outgrowth, the **ligule.** Describe its shape and state toward which end of the leaf it projects The slightly swollen base of the ligule is

inserted in a depression (**foveola**), smaller than the fovea and directly above it. This last point is not easily made out except with the aid of a hand lens or microscope.

(*n*) Make drawings as follows:

(1) A leaf-base, 20 mm. at greatest breadth, showing all points observed under 4, (*i*)-(*m*).

(2) A diagram, 40 mm. in greatest width, of an imaginary cross-section of the sporophyll, taken through the middle of the fovea.

(3) A diagram, 15 mm. in greatest width, of an imaginary median longitudinal section through the base of a sporophyll.

D. Asexual Reproduction:

1. Note that some of the sporophylls bear large spores (**megaspores**), and some small spores (**microspores**). Study any constant differences (*a*) in structure, (*b*) in position on the stem, between the **megasporophylls** and the **microsporophylls.** Define each of these terms.
2. How many megaspores does a **megasporangium** contain?
3. Measure the diameter of a megaspore in millimeters. Study and describe its shape under the low power (mounted in water), noting any surface marks. Explain the presence of angles of the spore.
4. Make a drawing of a megaspore, 20 mm. in diameter. Indicate the amount of enlargement.
5. Study microspores under high power, describing their shape and surface marks. Draw two or three to the same scale as the drawing of the megaspore. The number of microspores in each sporangium in *I. echinospora* is said to be from 150,000 to 300,000; of megaspores 150 to 300.

6. Why is *Isoetes* a **heterosporous pteridophyte?** Compare it with *Polypodium vulgare* in this respect.

The Gametophyte

The germination of the spores and the development of the male gametophyte from the microspore, and of the female gametophyte from the megaspore, are very difficult to follow, and will be omitted here. The structures of the gametophytes, and the process of sexual reproduction should be carefully studied in a text-book, and demonstrated by the instructor, if material is available.

E. Nutrition and Growth:

1. Is the gametophyte at any stage dependent upon the sporophyte? The sporophyte upon the gametophyte? (*Consult a text-book.*)
2. It is important to remember that:
   (*a*) *The microspore begins to germinate before it is set free*, dividing into two cells, a large one and a small one. The smaller cell constitutes the entire vegetative portion of the **male gametophyte.** The larger cell develops into an antheridium, consisting of four wall-cells, and four central cells. Each of the latter develops into a multiciliate, spirally coiled sperm, resembling those of the true ferns.
   (*b*) *The megaspore begins to germinate after it it set free.* It never develops chlorophyll-bearing tissues. In germination the nucleus divides into about 50 nuclei before any cells are formed. The cells begin to be organized about the nuclei, forming a small-celled tissue in the apex of the spore (where the three ridges meet), and a larger-celled tissue below. Archegonia then develop in the small-celled tissue, and the

larger-celled tissue serves to nourish the young embryo-sporophyte, that develops from the fertilized egg.

The archegonia are exposed for fertilization by the splitting of the wall of the megaspore along the ridges, but *the prothallus itself does not project beyond the walls of the spore.*

When the sporophyte begins to develop from the fertilized egg, it continues to grow, without any resting period until it is mature.

3. Diagram the life cycle of *Isoetes*, as directed for the fern (I, 11, p. 78). Let *MG* = male gametophyte; *FG* = female gametophyte; *s* = sperm; *e* = egg; *S* = sporophyte; *mi* = microspore; *mg* = megaspore.

Equisetum (HORSETAIL)

A. Classification:

Division IV. Calamophyta.

Class II. Equisetineæ.

Order. Equisetales.

Family. Equisetaceæ.

Genus. *Equisetum.*

Species. (*e.g.*) *arvense L.*

B. Habitat:

1. The field horsetail (*E. arvense*), is common along railway embankments, roadsides, and fields. It apparently prefers north-facing slopes, and has a great tendency to become weedy.

C. Naked-eye Characters:

1. *The Stem:*

   (*a*) Using herbarium specimens or alcoholic material, if fresh material is not available, observe the underground stem (**rhizome**), and the upright aerial branches.

   (*b*) Of the latter, observe two kinds: (1) non-green, unbranched, bearing only scale-leaves, and terminating in a prominent strobilus or **cone**; (2) green and branched, also bearing scale-leaves, but no cone.

   (*c*) Which kind of aerial branch appears first above ground in the spring? What advantage may this possess for the species?

   (*d*) Explain the physiological significance of the green color of the non-reproductive branches. Of what significance, in this connection, is their profuse branching?

(*e*) Describe the surface of the stem along the internodes, noting the presence or absence of ridges, the hardness (or otherwise), and the "feel" of the surface. To what are the last two characters due?

(*f*) If material of *Equisetum hyemale* (the "scouring rush") is available, it will be instructive to burn a portion of the stem in a Bunsen flame, and to examine the unburned portion under a microscope. The preservation of the cell-walls, uninjured by the flame, is due to the fact that they are impregnated with silica, taken up by the plant from the soil in the form of a silicate, and secreted by the protoplasm of each individual cell. It is the presence of the silica that made this species useful for scouring cooking utensils, and thus gave it its common name.

2. *The Leaves.*

(*a*) Describe the shape and character of the leaves; their arrangement on the stem (*i.e.*, opposite, alternate, or whorled).

(*b*) Do the leaves function in the work of photosynthesis? In what organ or organs is that function performed?

(*c*) To what, in the fern, are the branches of the vegetative part of the stem analogous? To what are they homologous? To what, in the fern, are the scales at the nodes analogous? To what are they homologous? Explain.

3. *The Roots.*

(*a*) Briefly describe their character and distribution. Does their distribution on the rhizome bear any constant relation to the point of origin of the aerial branches?

4. Make drawings showing all points observed under *C*, 1–3.

D. Asexual Reproduction:

1. *Vegetative Propagation.*
   (*a*) Describe the possibility of the multiplication of new individuals by the isolation of pieces of the rhizome.
2. *Reproduction by Spores.*
   (*a*) Sketch the strobilus or cone ($\times$ 3).
   (*b*) Make a cross-section of the cone at about one-third of the distance from the apex, and observe the central axis, and the manner in which the sporangiophores are borne.
   (*c*) Carefully dissect off a sporangiophore, and observe (1) its stalk; (2) its peltate (shield-like) top; (3) hanging from the under surface of the shield, the **sporangia.** How many sporangia on each sporangiophore? Examine several sporangiophores to see if the number of sporangia is constant. Describe the dehiscence of the sporangia.
   (*d*) Examine the spores under the microscope. Can you detect more than one size; *i.e.*, is *Equisetum* a homosporous or a heterosporous plant?
   (*e*) Describe the appendages (**elaters**), of the spores. How many on each spore? They are formed by a modification of the outer coat of the spore. Observe their behavior when breathed on at frequent intervals.
   (*f*) While the spores are morphologically homosporous, they give rise to diœcious gametophytes. Are they, therefore, physiologically alike?

(*g*) Since the spores have different sex-value, some giving rise to antheridial, others to archegonial prothallia, suggest the advantage of the hygroscopic elaters in tending to tangle up together several spores before they germinate.

(*h*) Make drawings illustrating all points observed under *D*, 2, (*a*)–(*f*).

E. Sexual Reproduction:

1. It is not essential in an introductory course to study the gametophytes, and sexual reproduction of *Equisetum* in the laboratory, and it is seldom possible to secure suitable material in sufficient quantity for a large class.
2. If material is abundant and time permits, the gametophytes may be studied, described, and sketched, noting especially color and general form, branching, rhizoids, archegonia, antheridia, and the diœcious habit.
3. From prepared slides further details as to archegonia, antheridia, eggs sperms, and fertilization may be studied, under the instructor's direction.

F. Division of Physiological Labor:

1. Write two or three paragraphs describing the division of physiological labor, (*a*) as between various vegetative processes, and (*b*) between the latter and reproductive processes. Give special attention in this to the work of each of the three kinds of branches.

G. Life Cycle:

1. Make a diagram, as previously for other forms, illustrating the life cycle of *Equisetum*.

Lycopodium (Club-moss)

A. Classification:

Division V. Lepidophyta.

Class I. Lycopodineæ.

Order. Lycopodiales.

Family. Lycopodiaceæ.

Genus. *Lycopodium.*

Species. (*e.g.*, *clavatum L.*)

B. Habitat:

1. Nearly all the species of *Lycopodium* prefer moist situations. They are widely distributed over the earth, in both hemispheres, from the torrid to the frigid zones, and commonly grow in shady or partly shaded places. A few tropical species are epiphytic. They generally prefer a substratum rich in humus or other organic matter.

The Sporophyte

C. Naked-eye Characters:

1. *General Features.*
   - (*a*) Note whether, or not, the plant is differentiated into root and shoot, and the latter into stem and leaves. If the stem branches, briefly describe.
2. *The Stem.*
   - (*a*) Describe the attitude of the stem (*e.g.*, erect, trailing). Does the tip of the stem turn up, or otherwise?
   - (*b*) Describe the mode of branching.

(*c*) Are there any specialized (*e.g.*, cone-bearing) branches?

(*d*) Is there a terminal bud? Lateral, or axillary buds?

3. *The Roots.*

(*a*) Does the stem bear roots only at its posterior end, or otherwise? Describe.

(*b*) Briefly characterize the roots.

4. *The Leaves.*

(*a*) State their manner of distribution on the stem.

(*b*) Describe an individual leaf.

5. Make drawings as follows: (1) of a portion of the stem, to show mode of branching, distribution of leaves and roots, and other general features, natural size; (2) a leaf ($\times$ 5).

D. Asexual Reproduction:

1. *Vegetative Propagation.*

(*a*) Describe the method of propagation by the annual **apical growth** of the stem.

(*b*) Does the species you are studying in the laboratory possess buds or bulbils that may fall away, and develop into new plants? If so, describe their distribution on the stem; their relation to leaves, etc.

2. *Reproduction by Spores.*

(*a*) Describe the location of sporangia, especially their relation to leaves. Are they borne in the leaf-axils or on the leaf-surface? If the latter, on which surface? What relation do they bear to the leaf-base? Is there more than one sporangium to each leaf?

(*b*) Are there special sporophylls? If so, how do they differ, in location and characteristics, from the foliage-leaves? Are they aggregated

in a cone? If so, what effect does the formation of the cone have on the further growth of the branch?

(*c*) Is *Lycopodium* a *homosporous* or a *heterosporous* plant?

(*d*) Describe a single sporangium, its mode of dehiscence, and the relative number (*i.e.*, few or many) of spores it bears.

(*e*) Into what do the spores develop?

(*f*) Make drawings as follows: (1) a cone (× 4); (2) a sporophyll, with sporangium (× 10); (3) under the low power a few of the spores, and (from younger sporangia) a few of the spore-tetrads.

THE GAMETOPHYTE

E. Sexual Reproduction:

1. The gametophyte of *Lycopodium* is rarely seen, and not readily obtained in artificial culture. Its laboratory study may be omitted in a beginning course, but the subject should be presented by lecture, or preferably studied from a text and then discussed in class, with demonstrations of preserved material.
2. Diagram the life cycle of *Lycopodium*.

Selaginella (Little club-moss)

A. Classification:

Division V. Lepidophyta.

Class II. Lepidodendrineæ.

Order. Selaginellales.

Family. Selaginellaceæ.

Genus. *Selaginella.*

Species. (*e.g.*) *caulescens* Spring (Syn. *amoena* Hort.)[1]

B. Habitat:

1. Various species of *Selaginella* are common in cultivation in greenhouses. In nature they usually grow in moist situations, usually preferring shade.

The Sporophyte

C. Naked-eye Characters:

1. *General Features.*

(*a*) If possible, observe plants of various species growing in greenhouses, noting their general appearance and habit (*e.g.*, erect, climbing, trailing).

2. *The Stem.*

(*a*) By breaking off a small piece from the end of your specimen, ascertain whether the stem is tough, brittle, fibrous, etc.

(*b*) Describe the mode of branching (*e.g.*, alternate, opposite, dichotomous (forked), etc.). Do the branches bear any relation to the leaves, *e.g.*, are they in leaf-axils?

[1] The species *S. caulescens* has exellent cones for class study (E. 2, (*a*), p. 164).

(*c*) Is the branch differentiated into regions? If so, briefly describe.

(*d*) Does the branch show a tendency to be dorsoventral? How do you determine this?

(*e*) Do you note, in entire plants, any indications of response to gravity, moisture, or the direction of light?

2. *The Leaves.*

(*a*) Describe their position on the stem. Is the flat appearance of the stem due to the leaves being opposite, or to the attitude they have assumed as they mature.

(*b*) Are the leaves differentiated into petiole, blade, etc.?

(*c*) Describe any apparent adjustment or arrangement resulting in the most favorable illumination of leaves.

(*d*) Describe any variations in the color of the leaves, and endeavor to account for it.

3. *The Roots.*

(*a*) Is the plant rooted in the soil?

(*b*) Do the roots branch? If so, describe.

(*c*) Are there any other roots besides those in the soil? If so, describe them, and their location, and suggest any advantage they may be to the plant.

(*d*) Make drawings to illustrate all characters observed, including (1) a portion of the leafy branch ($\times$ 2); (2) a leaf ($\times$ 10); (3) aerial roots, if any.

D. *Microscopic Characters:*

1. *The Leaf.*

(*a*) Mount an entire foliage-leaf in water or clearing fluid on a slide. Observe under the micro-

scope, using low and high powers, and describe all details of leaf-structure thus brought out, including

(*b*) The shape and arrangement of the *cells;* the color, size, and distribution of the plastids in the cells; any other cell-contents;

(*c*) Variations between marginal cells, those along the central axis, and those lying between. Account for any constant differences observed. Do you think any observed differences may be attributed to environment? Explain.

(*d*) Can you identify a tiny, membranous flap, the **ligule,** near the leaf-base? On which side of the leaf (dorsal or ventral) is it? *Be sure to examine both sides of the leaf in this connection.*

(*e*) Are there stomata? If so, describe their location.

(*f*) Make drawings sufficiently large to illustrate all points observed under *D*, 1.

2. *The Stem.*

(*a*) With the razor, make thin cross-sections of the stem, and mount them in water or clearing fluid.

(*b*) Is the stem differentiated into (1) epidermis; (2) vascular regions; (3) fundamental tissue; (4) cortex? If so, describe.

(*c*) Note the presence or absence of air-spaces.

(*d*) Compare the tissue-system of the *Selaginella* stem with those in the fern.

(*e*) Describe the distribution of xylem and phloem in the vascular bundle.

(*f*) Do you find indications of vascular bundles passing out to the leaves?

(*g*) Draw a section of the stem (× 20), to illustrate 2, (*a*)–(*c*).

E. Asexual Reproduction:

1. *Vegetative Propagation.*

(*a*) Describe any means of vegetative propagation disclosed by your observations, already made.

(*b*) If opportunity offers, the vegetative propagation of *Selaginella* may be experimentally demonstrated in the greenhouse or Wardian case.

2. *Reproduction by Spores.*

(*a*) Observe the "cones." How are they distinguished? Are they terminal on the main branches, or axillary?

(*b*) Draw (× 5):

(*c*) Place a small portion of a branch, bearing several mature cones, under a glass bell-jar, and after twenty-four to forty-eight hours observe the distance to which the spores have been projected. Note the two kinds, their relative number, and differences in color, etc.

(*d*) Carefully remove sporophylls (1) from near the base of the cone; (2) from the middle or above, and mount in water or clearing fluid, keeping distinct, on separate slides, those from the two regions.

(*e*) Note both megasporophylls, bearing megasporangia, and microsporophylls bearing microsporangia. How are they distinguished?

(*f*) Are the sporangia inserted on the leaf, or on the stem in the axil of the leaf? Compare with the other plants studied in this respect.

(*g*) Describe the structure of the walls of the sporangia.

(*h*) Carefully count and record the number of megaspores in one megasporangium. Is the number aways even?

(*i*) Carefully observe the megaspores under a high power, and endeavor to account for their shape.

(*j*) Make drawings to show all points observed under *E*, 2, (*a*)–(*h*).

(*k*) Make a study of the microsporophylls, microsporangia, and microspores, similar to those just made under *E*, 2, (*a*)–(*j*).

(*l*) The number of microspores is too large to permit of their being readily counted. Suggest any advantage to the plant in such a large number of microspores. Explain the cause of the difference in size. Suggest an advantage to the plant in the large size of the megaspore.

(*m*) Mount megaspores and microspores together and make drawings to show their *relative* sizes.

(*n*) Make drawings to illustrate all points observed under *E*, 2, (*k*).

F. Sexual Reproduction:

1. The gametophytes of *Selaginella* are not readily obtained in suitable form for study. If prepared slides are available, studies may be made of:
   (*a*) Archegonia and eggs.
   (*b*) Antheridia and sperms.

G. Comparisons:

1. Compare the relative prominence of the gametophyte and sporophyte in *Selaginella*. Compare, in this respect, with all the forms previously studied.
2. Compare the method of reproduction by spores in *Selaginella* with that in the forms previously studied. Why should *Selaginella* be considered

either higher or lower in the scale of life than those forms?

H. Life Cycle:

1. Make a diagram to illustrate the life cycle of *Selaginella*. Briefly state differences between this life history and that of the fern, and of *Anthoceros*.

Zamia floridana (A Cycad)

A. Classification:

Division VI. Cycadophyta (The Cycads).

Class II. Cycadineæ (Modern Cycads).

Order. Cycadales.

Family. Cycadaceæ[1]

Genus. *Zamia.*

Species. *Floridana DC.*

B. Habitat:

1. Most of the Cycadales occur only within the tropics, but two genera, *Zamia* and *Cycas*, are also subtropical. *Zamia* occurs in the United States only in Florida, where it is rather common, and in Porto Rico. It is frequently cultivated in greenhouses.

VEGETATIVE ORGANS

C. The Stem:

1. Briefly describe the stem, noting its general appearance, size, relation between its diameter and height, variations in diameter, character of the surface, its relation to the surface of the soil. Note the presence or absence of branches.

D. The Leaves:

1. Describe their arrangement on the stem, the nature of the blade (entire, divided, etc.), the color, and

[1] By some botanists the genera *Zamia*, *Macrozamia*, and *Dioon*, having both staminate and carpellate cones, are assigned to a separate family (Zamiaceæ), distinguished from the Cycadaceæ (in the narrower sense), which bear only the microsphorophylls in cones.

the presence or absence of a petiole. Describe accurately the **vernation** (condition in the bud), as shown by young leaves just unfolding.

2. Suggest any advantage to the plant of any of the facts recorded under *D*, 1.
3. Compare the character of the leaves with that of any of the ferns.

E. The Roots:

1. Briefly state their location and functions.

Reproductive Organs

F. The Staminate Cones:

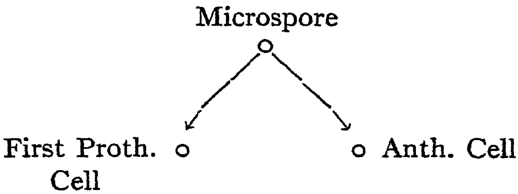
1. Describe their appearance, and, if the material is suitable, their distribution on the plant.
2. Describe, accurately, the distribution of the microsporophylls (**stamens**) on the main axis, or stem, of the cone.
3. Make drawings, natural size, showing (*a*) a surface view of the cone; (*b*) the cone as seen in longitudinal section.
4. Remove one of the stamens. Describe it, and note the microsporangia (**pollen-sacs**) attached to its lower surface. Describe them, their number, distribution, mode of attachment, and manner of opening (**dehiscence**).
5. Make drawings (*a*) of a stamen with pollen-sacs attached; (*b*) of two or three pollen-sacs ($\times$ 10).
6. The staminate cone is in reality a *primitive flower*. From this study, of what structure would you infer that flowers are a modification?

G. The Male Gametophyte:

1. The young **pollen-grain** (microspore) begins to germinate before it leaves the pollen-sac, two divisions of its nucleus taking place. By the first cell-

division two cells are formed, one, the **first prothallial cell,** representing the vegetative portion of the male prothallus; the second, or **antheridial cell** (called the "antheridial initial" by some botanists), divides again, forming a **tube-cell** and a **generative cell.** By the division of the generative cell, the **stalk-cell** and **body-cell** are formed. The division of the body-cell gives rise to two **sperm cells,** and each of these latter becomes transformed into a motile **sperm.**

2. Complete the following diagram of the above described sequence of cell-divisions:



3. Mount several pollen-grains in water, and examine them under the high power. Describe their shape and contents. Draw.
4. The study of the mature male prothallus, produced by the formation of a **pollen-tube,** will be omitted here.

H. The Young Carpellate Cone:

1 The youngest cones offered for study were collected about March 1.
2. Describe the cone, and the distribution of the megasporophylls (**carpels**) on its main axis, or stem. Draw, natural size.
3. Carefully dissect[1] off one of the carpels. Describe its form and surface characters, and note the num-

[1] For economy of material, with large classes, excised carpels may be supplied.

ber, and place, and mode of attachment of the large megasporangia (**ovules**). Note their color. Draw, natural size.

4. Do you find smaller, undeveloped ovules? These have probably not been pollinated.

I. The Young Ovule:

1. Is the ovule enclosed by the carpel, or is it naked? State why *Zamia* is classed as a **gymnosperm.**
2. At the end of the ovule, opposite its point of attachment, note the small, often slightly elevated, dark spot, which marks the place of the **micropyle** (small gateway), through which the pollen-grain passes in order to reach the pollen-chamber within. This process is called **pollination.** In *Zamia*, pollination occurs about Jan. 1.
3. Remove an ovule, carefully noting where and how it is attached to the carpel.
4. Describe its surface and shape. How may the latter, in part, be accounted for? At the end opposite the micropyle observe the scar (**hilum**), where the ovule was attached. Draw.[1]
5. With the scalpel, make a slight longitudinal incision of the **integument** (wall) of the ovule, being careful not to cut too deeply, so as to injure the delicate structures within. Describe the character of the tissue of the integument.
6. The tissue next within the integument is the **nucellus,** or megasporangium. The integuments are outgrowths of the nucellus.
7. After making a longitudinal incision, very carefully remove the nucellus, *noting the greater thickness of*

[1] Arrange all the drawings, showing the ovule at different ages, serially, on a new sheet of drawing paper, so as to facilitate the comparison of the different stages, and to show at a glance the changes which the different parts undergo.

the tissue at the micropylar end. This tissue serves as nourishment for the germinating pollen-grain.

8. Within the nucellus is the globular young female gametophyte, or **endosperm.** Describe and (from your reading in the text-book) account for its consistency.
9. With the razor make a median, longitudinal section of the entire ovule. Study and draw, naming all the parts.

J. Older Stages in the Development of the Ovule:

1. Examine ovules collected about April 1, as directed under *I*. Compare their size with that of the younger ovules. Observe the fleshy texture being assumed by the outer portion of the tissue of the integument, and the differentiation of a harder inner layer (**endopleura**).
2. Describe the changes which the nucellus has undergone. Account for these changes. (The appearance of the nucellar tissue may be due, in part, to its disintegration by the growing pollen-tubes.)
3. Remove the nucellus and describe the appearance of the endosperm. Note the slight depression in its micropylar end. What change has taken place in its consistency? Account for the change.
4. With a scalpel cut away the endopleura, and then, with a razor, make a clean, median longitudinal section of the endosperm, and observe, imbedded in its micropylar end, two or more **archegonia.** These open into the depression, mentioned above, by a short neck, composed of only two cells. The short **neck-canal** may be seen if the section passes through a suitable plane.

5. With a hand lens observe the wall of the archegonium, and within, filling the **venter,** the large ovum, or **egg.**
6. As outlined above (*J*, 1–4), examine and describe an ovule one month older (about May 1), carefully noting the changes which the different parts have undergone. Observe especially the development of the hard inner layer of the integument. May this feature be of any advantage to the plant? If so, how? Does any of the nucellus remain? If so, describe. Note, in the depression of the endosperm, *the openings into the archegonia.* How many are there? Draw this depression as seen in end view.
7. Construct a *diagram* of an ovule of this age as seen in median longitudinal section, carefully labeling all parts.
8. Make a diagrammatic drawing of a cross-section of the same ovule passing through the venters of the archegonia.
9. In ovules one month older (about June 1) the sac-like **proembryo** may be seen, lining the walls of the venter of the archegonium, and, growing from its basal end into the tissue of the endosperm, the prominent **suspensor,** at the free end of which the **embryo** begins to develop. As the suspensor and embryo increase in size, a cavity is formed in the surrounding endosperm. This cavity results from the *digestion of the endosperm tissue*, which goes to nourish the growing embryo and suspensor.
   Suggest how this digestion and subsequent nutrition may be accomplished.
10. Make a drawing of a median longitudinal section of the ovule at this stage.

K. The Seed:

1. The seed matures about July 1. Study the structure of a ripe seed, comparing it in every point with the structure of the unripe ovule, as directed above (*J*, 1–10).
2. Note the soft, outer layer of the integument. Describe it.
3. In cutting away the hard, shell-like inner layer (**endopleura**) be careful not to disturb the portion of the nucellus that fits like a cap over the mycropylar end of the endosperm. Now carefully lift this portion of the nucellus and observe the long, coiled suspensor attached to it, and (at its other end) to the projecting thick, round peg (**hypocotyl**) of the embryo.
4. With the scalpel gradually remove one-half of the endosperm, until you expose the embryo (**young sporophyte**) imbedded in it. Is the embryo curved or straight? Is it now confined to the venter of the archegonium?
5. Observe that the hypocotyl bears fleshy seed-leaves (**cotyledons**). How many are there? Compare their lengths.
6. Does more than one embryo come to maturity in any one seed?
7. When the sporophyte of *Zamia* begins to develop, is its growth continuous to maturity, or does a period of rest intervene between two stages of growth? Compare *Zamia* with the fern, moss, and *Selaginella* in this respect.
8. Define a seed, and state how it differs from an unripe ovule, and from a spore.
9. Suggest any advantage to the plant of the seed-habit.

L. Nutrition:

1. Describe the relative ability of the mature sporophyte and the gametophyte to lead an independent existence. Is the gametophyte ever independent of the sporophyte? Does the sporophyte ever live parasitically on the gametophyte?
2. Why does the embryo-sporophyte need a supply of food stored in the seed?
3. Would it be of any special advantage to *Zamia* to have a well-developed male gametophyte? Why?
4. At home write a detailed description of the changes undergone by a starch grain, formed in the leaf of a mature carpellate sporophyte, until it becomes part of the tissue of the embryo-sporophyte of the next sporophyte-generation.
5. Make a diagrammatic outline (using words, not drawings) showing the life cycle of *Zamia* from sporophyte to sporophyte.

Pinus Laricio (Austrian pine)

A. Classification:

Division VI. Spermatophyta (seed-bearing plants).

Subdivision A. Gymnospermæ (seeds not inclosed in an ovary).

Class I. Pinoideæ (pine-like plants).

Order. Coniferales (cone-bearing plants).

Family. Pinaceæ (pine family).

Genus. *Pinus* (the pines).

Species. *Laricio* var. *austriaca* Endl. (Austrian pine).

B. Habitat:

The Coniferales are widely distributed over the earth's surface, often forming extensive forests. The genus *Pinus* occurs in North America throughout Canada and the northern United States, from the Atlantic to the Pacific coasts. The white pine (*Pinus Strobus*) occurs from Canada south along the Alleghanies to Georgia, and west to Illinois and Iowa. The western yellow pine (*P. ponderosa*) extends south to western Nebraska, Texas, Mexico, and California. The long-leaved or "Georgia pine" (*P. palustris*) is found near the coast from Virginia to Florida, and Texas. The spruce-pine (*P. echinata*) also occurs as far south as Florida, and in Illinois, Kansas, and Texas. Considerable forests of it are found in southern Missouri. The loblolly pine (*P. tæda*) extends along the coast from Delaware to Texas and north up the Mississippi Valley to Arkansas. *Pinus Laricio* is not native to the United States, but has been introduced into cultivation, as has also *P. sylvestris*, the "Scotch pine," of northern Europe, and other species.

Pine wood was formerly one of the most valuable and at the same time one of the cheapest of soft-wood timbers, but owing to an utter disregard of the principles of scientific forestry, it is now one of the scarcest and most expensive. Regions that were formerly extensively forested, and the center of a prosperous lumber industry, are now waste land, often occupied by tall stumps, and a source of no profit. It is not necessary to destroy the forests in order to obtain an abundant supply of lumber, provided only that the crop be harvested in accordance with scientific principles. *The conservation of the forests is one of the most important economic problems confronting our country, and excellent opportunities are now offered for well-trained foresters.*

VEGETATIVE ORGANS

C. The Stem:

1. In *Pinus* the shoot, as usual, is composed of the stem and the leaves. The stem is divided into a main part, or **trunk,** and lateral branches. The entire portion of the shoot, except the trunk, is designated by foresters as the **crown**[1] of the tree.
2. The following observations (*C*, 3–10) of the stem as a whole are to be made out of doors, recorded at this place in the laboratory notes, and handed in at the next class period.
3. Designate the type of the trunk as **excurrent** (*i.e.*, extending, entire from the ground to the apex of the tree) or **deliquescent** (*i.e.*, extending entire for only a short distance from the ground, and then subdividing into the numerous limbs and smaller branches of the crown[1]).

[1] The use of the term *crown* in this sense is quite different from its older use by plant anatomists to designate the region (usually at or near the surface of the ground) where the root and shoot join.

4. Describe variations in the diameter of the trunk.
5. Do you find prominent swellings (**buttressing roots**) at the base of the trunk? If so, suggest their possible advantage to the tree.[1]
6. Describe the outline of the crown as flat, conical, or cylindrical.
7. Describe the appearance of the **bark.**
8. Note that the lateral branches *appear* to be given off in **whorls,** or circles, at regular intervals along the trunk. Observe closely and state whether these are true whorls (*i.e.*, the component branches in exactly the same horizontal plane), or pseudo-whorls (*i.e.*, the branches not really in the same plane).
9. Do you find enlargements on the under side of the larger limbs at the base? Suggest any advantage this may be to the limb; the conditions resulting in their formation.
10. Draw a diagram illustrating all points observed under *C*, 1–9. Make the trunk 15 dm. high.

D. The Vegetative Branches:

1. In specimens furnished, note the two kinds of vegetative branch: The main or "long" branch, bearing scale-like leaves, and, in the axils (upper angle made by the leaf with the branch that bears it) of these scales, the dwarf branches, bearing the foliage-leaves or pine "needles." In what does the long branch terminate?
2. *The Long Branch.*
   (*a*) Describe the arrangement (spiral) and distribution of the dwarf branches on the long ones.
   (*b*) Note the rings of dry bud-scales or scale-scars at intervals along the branch. The sections

[1] The conditions favoring the formation of buttresses should be discussed in class.

of the branch between these rings represent the amount of each year's growth in length. Ascertain the age of your specimen.

(*c*) State several ways in which younger portions of the stem may be distinguished from older.

(*d*) For how many years do the dwarf branches remain attached? The scales that subtend them? Observe the scars left by the fallen dwarf branches.

(*e*) Illustrate by a drawing the features observed under *D*, 2, (*a*)–(*d*).

(*f*) Measure and record the amount of each annual growth in length.

(*g*) Draw a horizontal line as many decimeters long as your specimen is years old. Mark off the line into decimeter spaces, and at the end of each decimeter erect a perpendicular as many millimeters high as the branch grew during the corresponding year. Connect the tops of these perpendiculars by a gently curving line, which will be the curve of annual growth in length for the period covered.

(*h*) Suggest reasons for the observed differences in amount of annual growth; for the direction of growth taken by the branch at various times.

(*i*) Make a diagram of a cross-sectional view of the branch, and describe the relative position of **wood, pith,** and **bark.** Does the bark contain any chlorophyll? If so, in what region is it found?

3. *The Dwarf Branch.*

(*a*) Do you find nodes and internodes, or any other evidence that these branches grow in

length each year? Find evidence that they do not.

(*b*) Compare the number of needles borne by each dwarf branch. Is the number constant? On what part of the branch are they borne?

(*c*) Note the bud-scales, some of which form a sheath about the bases of the needles.

4. *The Foliage-leaves.*

(*a*) Describe their shape. Are they differentiated into petiole and blade?

(*b*) Make a drawing, natural size, of an entire leaf, and a diagram (× 10) of a cross-sectional view.

5. *The Terminal Bud.*

(*a*) Describe its color, coverings (**bud-scales**), and shape. Draw (× 3).

(*b*) With the scalpel remove one of the bud-scales at its base. Describe and draw (× 5).

(*c*) Now remove, one at a time, the remaining bud-scales, having care not to break or injure the tender inner tissues.

(*d*) Describe the place and mode of attachment of the scales.

(*e*) Explain how they are adapted, in structure and position, to protect the bud. From what do they protect it?

(*f*) Describe the color of the inner tissues. Can *Pinus* form chlorophyll in the dark? Explain.

(*g*) Make a drawing (× 5) of the bud after the scales have been removed.

(*h*) With a sharp scalpel make a median longitudinal section of the bud. Observe the central, conical axis, bearing thin membranous scales. In the axil of each scale find a small knob-like outgrowth.

(*i*) Make a drawing (×10) of the longitudinal view.

(*k*) Into what will the bud develop? What will become of each of its parts?

(*l*) How much of your specimen represents last year's terminal bud? The bud of year before last?

(*m*) When the annual growth of a branch ends with the formation of a bud the growth is called **determinate.** Is the growth of the dwarf branches determinate or indeterminate? Of the long branches?

E. Homologies:

1. Organs which perform like functions are **analogous** to each other. Organs which correspond to each other structurally *i.e.*, which have the same morphological value, are **homologous.** For example, the flat, chlorophyllous stems of cacti and the foliage leaves of the maple tree are analogous, for they both function as organs of photosynthesis; but they are not homologous, for one is a stem, the other a leaf. The bud-scales of *Pinus* and the pine "needles" are homologous, *i.e.*, from the standpoint of structural value (morphological standpoint) they are both leaves. But they are not analogous, for, whereas the "needles" act as organs of photosynthesis, the bud-scales do not, as they have no chlorophyll.
2. One of the most important, and often more difficult, problems of morphology is correctly to interpret the structural value of an organ; in other words to recognize homologies; for any organ may be profoundly modified, and appear so disguised as to make it extremely difficult to recognize its morphological significance. *Pinus* furnishes an excellent

illustration of the modification of organs for various functions.

3. Enumerate all the **homologs** of the foliage-leaf found thus far on *Pinus*, and show why the organs you name are homologous.

Reproduction

F. *The Staminate Cone:*

1. On which portion of the vegetative branch are the **staminate cones** borne? Do they extend clear to the tip of the branch, *i.e.*, are they ever terminal? In what does the tip of the branch that bears them terminate? Ascertain their length and greatest diameter in millimeters.
2. Are the cones subtended by (*i.e.*, borne in the axil of) a scale-like leaf? Note whether they are sessile or stalked?
3. Observe the spiral-like arrangement of the **microsporophylls** of the cone.
4. The staminate cones are modified branches. To which of the vegetative branches are they homologous?
5. Make a diagram ($\times 2$) showing the mode of attachment of the cone and the subtending scale.
6. With a razor bisect a cone longitudinally and observe the central axis, bearing the microsporophylls, or **stamens.**
7. With the air of a hand lens, or dissecting microscope, observe the short stalk of each stamen and, on the under (dorsal) side of the broadened stalk, two small pouches, the **pollen-sacs** (microsporangia), containing **pollen-grains.**
8. Make a diagram ($\times 10$) of the cone as seen in longitudinal section.

9. Remove an entire stamen and observe that the tip of it is turned up so as to fit over the end of the stamen next above it. Suggest any advantage in this arrangement.
10. Make a drawing to illustrate this feature.
11. Make a cross-section of the stamen and ascertain of how many pollen-sacs it is composed. Draw. The pollen-sacs of the stamen constitute the **anther.**
12. The structure of the staminate cone shows it to be in reality a simple flower. It is homologous to the staminate flower of some of the higher plants. To what in *Zamia* is it homologous?

G. The Young Male Gametophyte:

1. Mount several mature pollen-grains in water and examine with the high power.
2. Observe the body of the grain, and the two lateral wing-like expansions, developed from the outer coat of the pollen-grain. Suggest their use.
3. Within the grain observe the **tube-nucleus** near the center, and the **generative cell** near the wall farthest from the wings. Look for the **prothallial cell,** which frequently may be seen between the wall of the grain and the generative cell.
4. Make a drawing, 25 mm. broad, showing all features observe under *G*, 1–3.
5. The nuclear and cell-divisions which give rise to these structures are steps in the germination of the microspore. Into what does the microspore of the heterosporus pteridophytes develop by germination? To what, then, in *Isoetes* or *Selaginella,* is the mature pollen-grain of *Pinus* homologous?
6. If prepared microscopic slides are available, more detailed study may be made of the structure of the pollen-grain.

H. The Young Carpellate Cone:

1. On which internode of the vegetative branch are the **carpellate cones** borne? On what part of the branch? Do they occur singly or in clusters? As terminal or as lateral outgrowths?
2. Note that each carpellate cone is borne at the tip of a stalk. Describe any outgrowths on this stalk.
3. Describe the attitude of the cone at the time of pollination, as erect or pendent.
4. Observe the spiral arrangement of the cone-scales, somewhat more marked than in the staminate cone. In fresh specimens the cone-scales are slightly separated from each other at the time of pollination. Explain the advantage of this.
5. Make a drawing ($\times$ 2) of the cone with the stalk that bears it.
6. Make a median longitudinal section of the cone and stalk, and represent by a drawing all parts seen.
7. Carefully dissect off one of the central cone-scales, being sure to note which is the inner (ventral) and which the outer (dorsal) surface of the scale, and observing the membranous bract which subtends it.
8. On the inner surface of the scale, near the base, observe with the hand lens two **ovules,** each with two little prongs, between which is the **pollen-chamber**; between and above the ovules a pointed outgrowth.
9. Make drawings ($\times$ 10) of the ovuliferous scale as seen (*a*) from the side; (*b*) from the outer surface, showing the bract; (*c*) from the inner surface, showing the ovules.
10. The ovules are megasporangia surrounded by a protecting integument.

11. There is some evidence for considering the **ovuliferous scale** and the bract that subtends it as a megasporophyll, or carpel. On the basis of this interpretation the bract would be homologous to the ligule in *Isoetes* or *Selaginella.* But other facts argue against this theory, and lead to different interpretations, so that the exact homology of the organ is in doubt. Possibly it represents two megasporophylls or carpels. If so, we must interpret the carpellate cone, not as a flower, like the staminate cone, but as inflorescence, or cluster of flowers, each scale representing a flower.

I. The Mature Male Gametophyte:

During the first spring pollination takes place, as described in the text-book, and the growth of the pollen-tube begins. Its growth is very slow, however, until the following spring, when the growth becomes more vigorous. The tube-nucleus passes to the tip of the pollen-tube, which penetrates the tissues of the nucellus (*I*, 6, p. 170), digesting a channel for itself as it grows, usually branching, and feeding on the digested tissue. The generative cell divides into a **body-cell** and a **stalk-cell,** and the nucleus of the body-cell again divides into two **sperm-nuclei.**

K. The Female Gametophyte:

Near the time of pollination the megaspore consists of one uninucleate cell (the one-celled stage of the embryo-sac). By repeated nuclear-divisions the nucleus of the megaspore gives rise to a large number of nuclei, which at first lie free in the surrounding cytoplasm; but later each of these nuclei organizes about itself a cell, surrounded by cell-walls. The tissue thus formed within the embryo-sac, and enlarged by growth, forms the young female gametophyte (endosperm). The

megasporangium, surrounding the endosperm, is called the **nucellus,** as in *Zamia*, and both these structures are surrounded by a protecting envelope, the **integument.** The pollen-chamber lies between the tip of the nucellus and the integument. The micropyle leads through the integument to the pollen-chamber. In the pollination of *Pinus* the entire pollen-grain passes into the pollen-chamber through the micropyle.

L. The Ovule:

The endosperm, nucellus, and integument together form the young ovule. Nearly one year is required for its development to the stage described above. In the second spring, while the pollen-tube is rapidly elongating, and the nuclear divisions noted above are taking place within it, several archegonia develop in the micropylar end of the endosperm. In the venter of each archegonium lies the large egg.

M. Fertilization:

Eventually the pollen-tube *enters the neck of an archegonium* (compare with the process in *Zamia* and other Cycads), its contents are discharged into the venter, and one of the sperm-nuclei fuses with the nucleus of the egg. Thus fertilization is accomplished, about one year after pollination. The transfer of the sperm-nucleus to the egg by means of a pollen-tube is called **siphonogamy,** and plants in which this occurs, **Siphonogamia.**

The one-year-old cone, to be studied next, represents the stage of development at about the time of fertilization. The sperms of *Pinus* are non-motile.

N. The One-year-old Carpellate Cone:

1. Compare the position on the branch, and the attitude of the one-year-old cones with that of the cones at the time of pollination.

2. Study these cones as directed above (*H*, 1–11), comparing the older and the younger organs. Endeavor to explain any differences observed.
3. Record the length and greatest diameter of the one-year-old cone, and make a drawing of it, natural size.

O. The Two-year-old Carpellate Cone:

1. Record, its position on the branch, its attitude, and dimensions. Compare it, in these points, with the young, and one-year-old cones. Draw, natural size.
2. Make drawings of a detached scale as seen from (*a*) the outer (dorsal) surface, (*b*) the inner (ventral) surface, (*c*) the side. Describe any changes observed in the appearance and relation of the various points.
3. **Note that the ovule has developed into a winged seed.**

P. The Seed:[1]

1. The seeds are usually shed from the pine cone during the third summer, about two years and a quarter after pollination.
2. Record the dimensions, shape, and character of the surface of the seed. The small depression in the smaller end of the seed locates the **micropyle,** which is now grown together. Draw, natural size.
3. Let fall from a height of several feet a seed of some species having wings still attached, and note the approximate time required to reach the ground. Remove the wing and repeat the observation. Suggest a use of the wing. Is it very firmly attached to the seed?

[1] The large seeds of the nut-pine, *Pinus edulis,* or of *Pinus pinea,* may advantageously be used for this study.

4. Remove the tough, outer seed-coat (**testa**), which is the mature **integument**, referred to in *K* and *L* (pp. 184–185). The integument is analogous to an indusium. Why?
5. Underneath the testa, observe the thin, membranous inner **seed-coat**, formed by a separation and differentiation of an inner layer of the tissue of the integument. Describe its color and surface-character as seen under the hand lens. Compare with *Zamia*.
6. Observe the small hole through the micropylar end of the inner coat. What does this represent?
7. Remove the inner seed-coat, having care not to disturb the brownish, membranous cap on the micropylar end of the kernel. This cap is the remains of the nucellus (megasporangium). Note the modification of its tissue at the place through which the pollen-tube passed on its way to the embryo-sac. The remainder of the nucellus was consumed by the female gametophyte during the development of the latter.
8. What is the homology of the white, fleshy kernel of the pine seed.
9. Make a drawing ($\times$ 4) of the endosperm and nucellus.
10. Remove the nucellar tissue. Is it firmly attached to the endosperm? Describe the appearance of the endosperm under the nucellar cap.
11. Very cautiously separate the endosperm into longitudinal halves. Begin the dissection at the end opposite the micropylar end so as not to injure the embryo-sporophyte within.
12. Observe that the embryo lies in a distinct cavity or chamber, its tissues being quite distinct, anatomi-

cally, from those of the gametophyte. Can you account for the formation of this cavity?

13. Note that the embryo is composed of a main axis, bearing a whorl of **cotyledons** borne near one end. Can you detect distinct regions of the axis? If so, how many, and how are they distinguished? How many cotyledons are there? Is the number always either odd or even?
14. Observe that the embryo is attached at the end opposite the cotyledons to a slender filament, the **suspensor.** At this end of the embryo-chamber may frequently be seen the disorganized remains of other embryos that failed to develop. In rare instances two embryos develop in one seed. This is called **polyembryony,** a condition very common in lemons, and other citrous fruits.
15. Make a drawing of the young sporophyte, 50 mm. long.
16. Make a median longitudinal section of the embryo, and observe that the portion of the axis below the cotyledons (**hypocotyl**) is encased in an outer, strongly developed **root-cap,** which completely encloses the hypocotyl. Note further that the cotyledons are borne on the hypocotyl. From its opposite end (**radicle-end**) the **tap-root** will develop. The hypocotyl is the first internode of the sporophyte. Where is the first node?
17. At the summit of the axis, above the cotyledons and surrounded by them, observe the conical **epicotyl.** It will develop into the second and subsequent internodes. Explain the meaning of the term epicotyl.
18. Construct three diagrams ($\times$ 5) showing (*a*) the entire seed in longitudinal section (the embryo not

sectioned); (*b*) a cross section of the seed, passing through the cotyledons and epicotyl; (*c*) a cross-section of the seed passing through the hypocotyl.

Q. The "Germination" of the Seed:

1. Observe specimens of seeds and seedlings representing various stages of germination.
2. Describe (*a*) the changes that the various parts of the seed undergo, in shape, size, and position; (*b*) the manner in which the seedling breaks through the surface of the soil, and the advantage of this; (*c*) the relative rate of early growth of the root and shoot, and the significance of this; (*d*) color-change in the cotyledons, its significance and whether or not it can take place in darkness; (*e*) the fate of the endosperm, and the evident rôle of this tissue; (*f*) the manner of shedding the seed-coat; (*g*) the place of development and the character of any new organs. Draw.
3. Compare the germination of a seed, with that of a spore. What, in reality, is the germination of a seed?

R. General Questions:

1. To which alternating generation does the pine tree belong?
2. In a well-worded paragraph compare the relation of gametophyte and sporophyte in the moss, fern, *Isoetes* (or *Selaginella*), and *Pinus*.
3. State the relative prominence of the sexual and asexual generations in plant-groups of successively higher organization.
4. When the young sporophyte of *Pinus* begins growth does it grow continuously to maturity, or does a period of rest intervene? Compare it with *Isoetes* or *Selaginella* in this respect.

5. What changes would result in the formation of a seed in *Isoetes?* What interferes with seed-formation in that group? What changes would interfere with seed-formation in *Pinus?*
6. Define a seed. State several advantages to the plant of the seed-habit.
7. To what, in the fern, is the endosperm of the pine seed homologous? To what in the moss? To what, in *Isoetes* or *Selaginella*, is the pollen-grain homologous? To what in the fern? State, with reasons, whether the leaves of the moss-plant are homologous or analogous (or neither) to pine needles. In like manner, compare the organs of fixation of the moss-plant, of the fern-plant, of the fern prothallus, and of the pine tree.
8. Diagram the life cycle of *Pinus* as directed for Isoetes (*E*, 3, p. 153), substituting *pg*(=pollen-grain) for *MG*(=male gametophyte), and *es*(=embryo-sac) for *FG*(=female gametophyte).

Trillium (Wake-robin)

A. Classification:

Division VII. Spermatophyta.

Subdivision B. Angiospermæ (seeds enclosed in an ovary).

Class I. Monocotyledoneæ (embryo with one lateral cotyledon).

Order. Liliales (lily order).

Family. Liliaceæ (lily family).

Genus. *Trillium.*

Species.[1] (*e.g.*) *sessile L.*

B. Habitat:

All species of *Trillium* occur in the woods in early spring, and the genus has a geographic range extending from Nova Scotia westward to Manitoba, and southward as far as Florida.

[1] Any species of *Trillium* may be used, with minor changes in the directions; or, in fact, any other convenient genus of the Liliaceæ.

Note.—There are nearly 25,000 different speceis of Monocotyledons. The order of Liliales comprises about 5,000 species. The lower Monocotyledons have naked flowers (*i.e.*, no sepals and petals), with the parts **spirally** arranged, as in the Gymnosperms. The higher ones have the parts of the flower arranged in concentric circles or **cycles,** five in number (**pentacyclic**), with usually three members in each cycle. Our knowledge of the monocotyledon is not yet adequate to make possible a satisfactory classification. Taxonomists differ in various points. The authors of Gray's "New Manual" (7th Edition, 1908) subdivide the Liliaceæ into **Tribes.** Trillium is in the tribe *Parideæ.* Britton ("Manual of the Flora of the Northern States and Canada") and others, divide the Liliaceæ, as given in Gray, into four or more families, the trilliums being in the *Convallariaceæ,* or Lily-of-the-valley family.

C. The Shoot:

1. *General.*

   (*a*) Note its division into a main, thickened underground part (**rhizome**), bearing numerous roots, and a long slender aerial branch.

2. *The Rhizome.*

   (*a*) Describe its attitude (horizontal or erect), and its general appearance. Compare *Trillium* with the fern in this respect.

   (*b*) Are there branches, besides the aerial branch?

   (*c*) Note the thin membranous scales near the apical end. Record their number and position. Carefully remove them with the scalpel. What purpose may they serve? What is their homology? Make a drawing of one (× 1).

   (*d*) Observe the **nodes** and **internodes.** What do the nodes represent? Note the remnants of the old scales at each node. Compare the lengths of the internodes. What is the meaning of this?

   (*e*) Describe any other scars on the rhizome. Are they on nodes or internodes? What do they represent?

   (*f*) What develops each year at the growing end?

   (*g*) A plant that is continued indefinitely, from year to year, by means of a persistent root or stem, or both, is a *perennial:* one that persists for two years only, setting seed and dying at the end of the second season is a *biennial;* plants that set seed and perish at the end of one season are *annuals*. Name illustrations of each of these three classes of plants.

   (*h*) State, with reasons for your opinion, the age of your specimen.

3. *The Aerial Branch.*
   (*a*) Describe its general appearance, shape, length (compare several different specimens), coloration (color-pattern), and presence or absence of branches.
   (*b*) At which end of the rhizome is it borne? Is it a terminal or a lateral outgrowth? Is it an **axillary** organ (*i.e.*, borne in the axil of a leaf), or not? On a node or an internode?
   (*c*) Make a drawing 20 mm. in diameter, showing the distribution of the **fibro-vascular bundles** as seen in cross-section.
   (*d*) What does the branch bear at its summit? Do you find any exceptions to this?

The Roots:

1. Describe their distribution on the root-stalk. Do they occur on both nodes and internodes? State the significance of the observed distribution.
2. Record the presence or absence of branching.
3. Compare the appearance of new roots with that of older ones. On what part of the rhizome are they borne?
4. Describe the surface appearance of older roots. Remove a root 3 to 4 cm. long, hold it by each end? and gently pull (not hard enough to break the root). How does this affect the surface appearance? How do you think the original feature was produced?
5. With the scalpel cut a root squarely off near its base and observe the cross-sectional view. Distinguish three tissue-systems: (*a*) the **epidermis**; (*b*) the **central cylinder**; (*c*) between (*a*) and (*b*), the **cortex.**
6. Peel down a strip of the epidermis, and observe whether the wrinkling is confined to it or not.

7. Make a drawing, twice natural size, showing the external features of the rhizome, together with a portion of the aerial branch, and roots.

E. The Young Terminal Bud:[1]

1. Carefully remove the aerial branch and surrounding scales and observe the terminal (**apical**) bud.
2. Describe its color, shape, attitude, and the relation between it and the base of the aerial branch. Draw (× 2).

F. The Mature Terminal Bud:[1]

1. Use material gathered in later autumn, and carefully dissect away the outer bud-scales. Identify the parts found within.
2. Make careful drawings, and interpret the significance of this observation.

G. The Foliage-leaves:

1. Describe the number, location, and arrangement of the foliage-leaves. Are they **petiolate** or **sessile?**
2. Observe:
   (*a*) The **coloration** (described, for *T. sessile*, as "blotched").
   (*b*) The outline of the **base, apex,** and **margin.**
   (*c*) The **venation.**

H. The Flower:

1. On what part of the shoot is it borne? Record the presence or absence of a flower-stalk (**peduncle**). Explain the significance of the specific name of this species (*T. sessile*). Has the flower an odor? If so, describe it.
2. Observe:
   (*a*) The outer circle of parts (**calyx**) composed of separate **sepals.** How many sepals are

[1] On account of the large amount of material required, it is desirable to make *E* and *F* class demonstrations by the instructor.

there? Describe them as you did the leaves. NOTE.—The term calyx comes from the Greek word *kalyx*, a cup; the verb is *kalypto*, to cover. What organ of the moss has its name derived from the same source as calyx?

(*b*) The circle of parts (**corolla**) next within the calyx, composed of separate **petals.** How many petals? Are they opposite or alternate with the sepals? Record their color in fresh (not preserved) specimens. Describe a petal as you did the leaf and sepal.

(*c*) With the corolla, a circle of three microsporophylls (**stamens**) each opposite a sepal. By carefully bending back (but not removing) the sepals and petals, observe whether or not the other stamens are in the same circle as the first ones, or in an inner (higher) circle. Describe their location with reference to the petals. Record the total number of stamens. Note that each stamen is composed of:

(1) A stalk (**filament**), bearing at its tip,

(2) An **anther,** composed of marginal, linear **pollen-sacs** (microsporangia), and connecting tissue (the **connective**). Observe whether the connective is prolonged beyond the sporangia. Note that the pollen sacs **dehisce** (open) on the inside (*i.e.*, are **introrse**). Describe their manner of dehiscence.

(3) What do the pollen-sacs contain? Describe its color.

(*d*) The central **pistil** composed of:

(1) The basal ovule-case (**ovary**). How many angles has it? How many lobes?

(2) The relatively long **styles.** How many? Their inner surface is modified into

(3) A **stigma.** Describe this stigmatic surface as seen both with unaided eye and under the low-power. Usually, numerous pollen-grains may be seen adhering to it. What process has therefore taken place?

(4) The ovary is thus seen to be, not simple, but **compound.** It is composed of three **carpels,** bearing ovules. Since the ovules are megasporangia, what is the homology of the carpels?

3. Look up in the dictionary, and record at this point, the derivation of the names of the various parts of a flower, studied above.

(*a*) It is important to note: (1) That the various cycles are each composed of the same number of parts; (2) that the parts of the various cycles alternate with each other.

(*b*) When the other organs of a flower are inserted below the pistil they are said to be **hypogynous.** Is this true of *Trillium?*

(*c*) The more or less enlarged end of the stem (or, in non-sessile forms, of the peduncle) on which the organs of the flower are inserted, is the **receptacle.**

4. Make drawings as follows:

(1) A sepal (× 1); a petal (× 1); a stamen (× 4) (both dorsal and ventral views); an imagined cross-section of an anther taken through the middle (× 4); the pistil (× 4); an imagined cross-section of the pistil (× 4).

(2) A **ground-plan** of the flower, 5 cm. in diameter, first drawing five equidistant concentric cir-

cles, and filling in the plan as directed by the instructor.

(3) An imaginary longitudinal section of the flower ($\times$ 2).

5. Compare the length of the stamens with that of the pistil. Carefully consider and state the relative probabilities of **self-pollination** and **cross-pollination.**

I. Non-sexual Reproduction:

1. Non-sexual reproduction in *Trillium* is confined to the growth of the persistent, underground rhizome. This organ is thick and fleshy, serving for the storage of food.

K. Sexual Reproduction:

1. *Microspores.*

(*a*) Mount in clearing fluid (or water) on a slide some of the pollen from an anther.

(*b*) Observe (first under low, then under high power) the individual pollen-grains. Describe their color and shape, and note the network of ridges on the surface of each.

(*c*) By carefully focusing on individual grains there may readily be detected in some of them one nucleus, in others, two. Those in the one-nucleate stage are mature **microspores.**[1]

2. *Male gametophyte.* The dividion of the microspore-nucleus is the first stage in the germination of the spore. To what do microspores, when they germinate, give rise? What, therefore, is the homology of the bi-nucleate pollen-grain?

(*a*) The larger nucleus is the **tube-nucleus,** and presides over the development of the pollen-tube. The smaller is the **generative nucleus.**

[1] Is the microspore gametophytic or sporophytic? Cf. Foot-note, p. 75.

(*b*) Make drawings, 2 cm. in diameter, of a microspore and of a male gametophyte, labeling all parts.

(*c*) After pollination, the formation of the **pollen-tube** takes place. This is usually spoken of as the "germination of the pollen-grain."

The tube emerges through one of several weak places in the wall of the grain, grows down through the tissues of the style, digesting its channel as it proceeds, or, in some species, following a canal already formed through the style. The **generative cell** (generative nucleus with its own protoplasm) follows down the pollen-tube, and divides into two non-motile **sperm-cells.** In some species, *e.g.*, *Sambucus* (elder), this division occurs before the tube develops. The pollen-tube passes through the micropyle, and discharges the sperm-cells near the egg. One of the sperm-nuclei fuses with the egg-nucleus, thus effecting **fertilization.**

(*d*) For convenience in handling material the observation of the finer structure of the anther and pollen will be deferred until after the microscopic study of the ovary and ovules (3, below).

3. *Megasporophylls* and *Megasporangia*. With a sharp scalpel or razor make a median cross-section of the ovary and observe:

(*a*) Its outline. Each of the lobes represents the section of one of the **carpels** (megasporophylls), which together compose the **tri-carpellate, compound ovary.**

(*b*) The number of compartments ("cells"). Do the **septa** (walls) that separate them meet in

the center? Compare the number of cells with the number of carpels.

(*c*) The **placentæ** (sing., *placenta*), or surfaces of the septæ to which are attached.

(*d*) The **ovules.** Do the ovules lie in a vertical or in a horizontal plane? Are they few or numerous? In *Trillium* the ovules are borne on **parietal placentæ.**

(*e*) The **funiculus** (stalk) by which the ovule is attached to the placenta. Observe (using magnifier that the ovules have curved through 180°, bringing their apical (micropylar) ends to their base or point of attachment to the funiculus. They are thus **anatropous** ovules.

(*f*) Make a drawing, 4 cm. in diameter, showing the ovary (and ovules) as seen in cross-section.

4. *Histology of the anther.* Development of the pollen.

(*a*) Use prepared slides. The sections on these slides are triple-stained with safranin, gentian-violet, and orange. By this means the various parts of the cell are given different colors, the cytoplasm a grayish tinge, the nucleolus and **chromatin threads** in the nucleus red, starch grains a deep blue.

Using slides showing the microspore-mother-cell stage of *Lilium canadense*, or other convenient plant,

(*b*) Observe the outline of the section as a whole.

(*c*) The central portion is the **connective,** containing a **vascular bundle.**

(*d*) Opposite the connective may often be seen a cross-section of the filament, with its vascular bundle.

(*e*) The numerous, conspicuous blue-stained bodies are **starch grains.**

(*f*) Note the **epidermis,** one cell thick. Does it cover the entire surface? Does it contain starch grains? Find numerous **stomata,** each with a small, underlying air-space.

(*g*) At each side of the section will be seen two **sporangia,** containing the large **microspore-mother-cells** (pollen-mother-cells), with prominent nucleus. Observe the network of **chromatin** within each of these nuclei. The mother-cells adhere more or less closely, depending upon age. Their final separation from other cells, and from each other, marks the first separation of the gametophytic from the sporophytic generation. *The mature microspore-mother-cell* is the first stage in the development of the male gametophyte (*Cf.* 6 (*a*), below, p. 202).

(*h*) Around the mother-cells note a layer of elongate cells, radially arranged, and with nuclei more or less disorganized. These are the tapetal-cells. Together they form the **tapetum.**

(*i*) Between the tapetum and epidermis lie the middle layers of cells forming the wall of the sporangium. How many cells thick is it?

(*k*) Make a drawing 8 cm. in longest measure, showing all features observed under 4, (*a*)–(*k*).

(*l*) By two successive divisions each pollen-mother-cell forms four **microspores** (young pollen-grains). They thus arise **in tetrads.**

(*m*) The more advanced disorganization of the tapetal-cells, including the breaking down of their cell-walls, and the *fragmentation of their*

nuclei into two or more. These cells serve to nourish the spore-mother-cells.

(*n*) The microspores. Do they lie free or connected? Describe their shape, surface-features, and number of nuclei.

(*o*) State again the first stage in the germination of the microspore. What is the resulting structure? What is its homology?

5. *Megasporangia; megaspore-mother-cell.*

Using prepared slides showing the megaspore-mother-cell, observe:

(*a*) The outline of the ovary as seen in cross-section.

(*b*) The presence or absence of an **epidermis**; of **stomata.**

(*c*) The **"cells"** of the ovary, each containing, in the section.

(*d*) Two young **ovules** (megasporangia). Are the ovules straight or curved?

At this stage the tissue of the ovule is chiefly **nucellus,** but soon there develops at the base of the ovule, outside of the nucellus.

(*e*) The **inner integument,** which grows up around the nucellus, leaving at the summit only a small passage,

(*f*) The **micropyle.** Outside the inner integument there usually develops.

(*g*) An **outer integument.** In anatropous ovules the development of the outer integument wholly or partially fails on the side where the funiculus adheres to the ovule. At the summit of the nucellus (apex of the ovule) is seen the large

(*h*) **megaspore-mother-cell** (embryo-sac-mother-cell), with very prominent nucleus and nuclelous.

(*i*) Make a diagram of all that you have observed under 5, (*a*)–(*h*), filling in the details for one ovule.

6. *Development of the Megaspores.*

(*a*) Like the microspore-mother-cell, the megaspore-mother-cell of *Trillium* divides twice, giving rise to four megaspores (**tetrads**), but *only one of these megaspores develops a gametophyte.* This spore enlarges, and by three successive divisions gives rise to an eight-nucleate female gametophyte, the **embryo-sac.** Three of these nuclei organize **antipodal cells** at the basal end of the embryo-sac, and three of them organize cells at the micropylar end. One of the latter is the **egg,** the other two the **synergids.** The two remaining nuclei fuse near the center of the embryo-sac, forming the **endosperm-nucleus** (sometimes called *definitive nucleus*), but the endosperm does not develop until after the fertilization of the egg.

(*b*) Unlike the microspores, the megaspores, in angiosperms, never become free, independent cells, but always retain an intimate physiological connection with the sporophyte of the next preceding generation (*cf.* 4, (*g*), above).

7. *The Embryo-sac.*

(*a*) Using prepared slides, study the embryo-sac in its two- to eight-celled stages, identifying the cells mentioned in 6 above. Draw.

L. Development of the Embryo:

1. After fertilization (*K*, 2, (*c*), p. 198), the oösperm develops the embryo-sporophyte, and while this process is taking place, the endosperm-nucleus, in many cases, fuses with the second sperm-cell

(double fertilization). By successive divisions the endosperm-nucleus develops into endosperm, which surrounds the embryo and will serve to nourish it when it re-awakens, at the "germination" of the seed.

M. Fruit and Seed:

1. Meanwhile, as a result of fertilization, the ovary resumes growth, and develops into a **fruit** (ripened ovary), while the ovule enlarges and undergoes numerous changes, ripening into a **seed.**
2. In a concisely worded paragraph, tell what a seed is, stating to which of the alternating generations the seed-coats, endosperm, and embryo belong.

N. Nutrition:

1. Discuss the nutrition of both the gametophyte and sporophyte of *Trillium*, as suggested above (*L*, 1–4, p. 174) for *Zamia*.

O. Tabular Review:

Fill in the tables below (pp. 204 and 205), by placing an *x* in the proper space, then state, in a well-worded paragraph for each table, what may be learned by an inspection of it.

TABLE III.—COMPARISON OF GAMETOPHYTES

Plant	Respires	Photosynthesises	Has stomata	Takes nourishment from the soil	Has conducting tissues	Can exist independently of the sporophyte	Partly parasitic on the sporophyte	Wholly parasitic on the sporophyte	Monœcious	Diœcious	Capable of asexual propagation	Has both vegetative and reproductive functions	Vegetative functions reduced
	1	2	3	4	5	6	7	8	9	10	11	12	13
Riccia													
Anthoceros													
Sphagnum													
Polypodium													
Equisetum													
Lycopodium													
Selaginella													
Isoetes													
Zamia													
Pinus													
Trillium													

		Riccia	Anthoceros	Sphagnum	Polypodium	Equisetum	Lycopodium	Selaginella	Isoetes	Zamia	Pinus	Trillium
Seeds inclosed in an ovary	19											
Seeds not inclosed in an ovary	18											
Produces seeds	17											
Produces no seeds	16											
Heterosporous	15											
Homosporous	14											
Vegetative functions predominate	13											
Reproductive functions predominate	12											
Capable of asexual propagation	11											
Wholly parasitic on gametophyte	10											
Partly parasitic on gametophyte	9											
Can exist independent of gametophyte	8											
Has conducting tissue	7											
Takes nourishment from the soil	6											
Has stomata	5											
Has chlorophyll	4											
Respires	3											
Passes through an embryonic to a more highly developed stage of maturity	2											
Never passes beyond an essentially embryonic stage	1											
Plant												